Innovative Food Science and Emerging Technologies 27 (2015) 1–14

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Inactivation of microorganisms by high isostatic pressure processing in complex matrices: A review

E. Georget [a,b,*,1], R. Sevenich [c,1], K. Reineke [d], A. Mathys [a], V. Heinz [a], M. Callanan [e], C. Rauh [c], D. Knorr [c]

[a] German Institute of Food Technologies (DIL), Prof.-von-Klitzing-Str. 7, 49610 Quakenbrück, Germany
[b] Leibniz Universität Hannover, Institute of Food Chemistry, Callinstraße 5, 30167 Hanover, Germany
[c] Technische Universität Berlin, Institute of Food Biotechnology and Food Process Engineering, Königin-Luise-Straße 22, 14195 Berlin, Germany
[d] Leibniz Institute for Agricultural Engineering Potsdam-Bornim e.V., Max-Eyth-Allee 100, 14469 Potsdam, Germany
[e] Nestlé Research Center, Route du Jorat 57, Vers-Chez-Les-Blanc, 1000 Lausanne, Switzerland

ARTICLE INFO

Article history:
Received 17 September 2014
Accepted 30 October 2014
Available online 10 November 2014

Editor Proof Receive Date 17 November 2014

Keywords:
High pressure processing
Food preservation
Sterilization
Pasteurization
Water activity
Microorganism inactivation

ABSTRACT

The benefits of high pressure processing (HPP) for microbial inactivation in food production include reduced thermal treatment and minimized effects on sensory and nutritional profiles. These benefits have resulted in increasing commercial production of high pressure pasteurized foods. In this review, the current state of the art in terms of vegetative cell and bacterial spore inactivation by HPP in complex food matrices is assessed with an emphasis on mechanisms of inactivation and treatment of products that have low or non-uniform water activity (a_w) profiles. Low a_w can be the result of a high concentration in solutes, the presence of oils/fats, or the physical removal of water through dehydration. Microbial inactivation in low a_w environments remains a particular challenge for HPP and studies on microbial inactivation observed in the different types of low a_w food matrices are reviewed in detail.

Industrial relevance: HPP-treated food products with low a_w have been on the market since the nineties, but the mechanisms of microbial inactivation at low a_w are still not well understood, which hinders the development of new applications in low or inhomogeneous a_w food. This review summarizes the state of the art in terms of HPP microbial inactivation mechanisms in model systems and various low a_w food environments. Thereby, it identifies existing and potential new applications as well as the current gaps and future research needs.

Contents

1. Introduction

High pressure processing (HPP) is a non-thermal processing technology of food which was first investigated in the late nineteenth

* Corresponding author at: German Institute of Food Technologies (DIL), Prof.-von-Klitzing-Str. 7, 49610 Quakenbrück, Germany. Tel.: +49 5431 183 0; fax: +49 5431 183 114.
E-mail address: e.georget@dil-ev.de (E. Georget).
[1] Authors with equivalent contributions.

http://dx.doi.org/10.1016/j.ifset.2014.10.015
1466-8564/

century by Hite (1899). Following this, early work focused on understanding the biological effects of high pressure on food microorganisms and how this could open opportunities for food preservation (Hoover, Metrick, Papineau, Farkas, & Knorr, 1989). Since then, a significant research effort has enabled the development of this tool and its transfer to the industrial-scale. To date, it has been mainly applied in the food industry as a post-packaging pasteurization step allowing for vegetative microorganisms inactivation. HPP provides a gentle pasteurization method in comparison to conventional thermal processing with minimal effects on sensory and nutritional profiles (Heinz & Buckow, 2010). The inactivation of bacterial spores by HPP has also been investigated and high pressure high temperature sterilization could produce uniform, minimally processed foods of higher quality (Mathys, 2008) than heat treatment alone (Georget et al., 2013). It has, however, not yet been successfully introduced into the food industry, possibly due to limited knowledge regarding the inactivation mechanisms of high resistant bacterial spores as well as technical limitations (Reineke, 2012).

A common element to all decontamination (pasteurization or sterilization) strategies by HPP is the need to account for the food matrices hosting these microorganisms. Food matrices are complex environments which may offer shelter to microorganisms, even under harsh treatment conditions. Specifically, low water activity (a_w) matrices have been shown to be particularly challenging to achieve microbial decontamination by any kind of decontamination strategies, including HPP (Doona & Feeherry, 2007).

However, low a_w products do not constitute a homogeneous category and the low a_w can result from different compositions or properties. The a_w in a food matrix can be influenced, locally or overall, by a high concentration in solutes, the presence of oils/fats, or the physical removal of water through dehydration. As a result, inactivation challenges in low or inhomogeneous a_w environments might be associated with different, and possibly complementary, mechanisms.

In this work, the current state of the art in terms of microorganism inactivation by HPP in complex matrices is reviewed. First, the application and limitations of high pressure processing for food preservation are presented. Then, high pressure inactivation of microorganisms in food matrices with low or variable a_w is introduced through 1) the impact of different solutes and resulting microorganism protection against HPP inactivation, 2) the impact of dehydration of food systems on the a_w and resulting microorganism protection against HPP inactivation, and finally 3) the impact of fats and oils on local a_w in food matrices and resulting effect on microbial inactivation by HPP.

2. Application of high pressure processing for preservation and current limitations

2.1. Vegetative microorganisms' inactivation by HPP

The use of HPP to inactivate pathogenic or spoilage vegetative microorganisms has been largely investigated for the pasteurization of commercial products for decades (Heinz & Buckow, 2010). Hite was the first to conduct experiments with high pressure in combinations with foods to extend shelf life in 1899, and reported that milk stayed sweet longer after the treatment with high pressure (Hite, 1899). Since then, significant research effort has focused on understanding the underlying mechanisms of the inactivation of microorganisms under high pressure conditions. HPP offers a lower thermal input into the product by comparison with conventional thermal treatment and therefore increases the quality of the food while maintaining food safety (Balasubramaniam, Farkas, & Turek, 2008; Barba, Esteve, & Frígola, 2012; Bermúdez-Aguirre & Barbosa-Cánovas, 2011; Bolumar, Georget, & Mathys, 2014; Hogan, Kelly, & Sun, 2005; Smelt, 1998). Despite the steadily increasing commercial production of high pressure pasteurized food with more than 500,000 t/y (Tonello Samson, C. 2014, Hiperbaric, Spain, personal communication), some important scientific and technological questions are still unresolved.

One of these issues is the impact of different intrinsic and extrinsic factors on the inactivation mechanisms of vegetative bacteria and bacterial spores under pressure. To unravel the impact of the different pressure and temperature combinations on a possible cell death or recovery, detailed analyses about the physiological state of the cells and how they are influenced by different food constitutes are needed. According to Le Chatelier's principle in a system facing a shift of equilibrium, all cellular components are affected by high pressure, including the cell membrane and its membrane proteins, enzymes and ribosomes as well as all the cell metabolism (Heremans, 2002; Smelt, Hellemons, & Patterson, 2001; Winter & Jeworrek, 2009). In general, prokaryotic cells show a higher resistance towards pressure than eukaryotic cells. Yeast and molds are in general more pressure sensitive although ascospores of some molds such as *Byssochlamys* and *Talaromyces* can be very pressure-resistant (Chapman et al., 2007; Considine, Kelly, Fitzgerald, Hill, & Sleator, 2008; Smelt, 1998). Within prokaryotes, gram positive microorganisms such as *Bacillus*, *Listeria*, *Staphylococcus* and *Clostridium* have a thicker peptidoglycan layer and are therefore generally more pressure resistant than gram-negative microorganisms (Considine et al., 2008; Dumay, Chevalier-Lucia, & Lopez-Pedemonte, 2010; Smelt, 1998).

The mechanisms leading to cell death have been investigated in several bacterial species (Huang, Lung, Yang, & Wang, 2014). However, the particular events leading to inactivation are not well understood (Buckow & Heinz, 2008; Cheftel, 1995; Klotz, Manas, & Mackey, 2010). High pressure between 300 and 800 MPa at ambient temperatures can lead to the unfolding and denaturation of important cell enzymes and proteins in vegetative microorganisms (Knorr, Reineke, Mathys, Heinz, & Buckow, 2011; Rastogi, Raghavarao, Balasubramaniam, Niranjan, & Knorr, 2007), but the specific pressure effects on microorganism are more complex and several different mechanisms leading to cell death can occur simultaneously when high pressures are applied. Primarily, pressure at a sufficiently high level, can induce enzyme inactivation, membrane protein denaturation and cell membrane rupture caused by a phase transition of the membrane and change in its fluidity (Abe, 2013; Ananta, Heinz, & Knorr, 2005; Winter & Jeworrek, 2009). The pressure level needed to achieve a 5 $\log_{10}$ reduction of pathogenic microorganism in different food-products ranges from 300 to 800 MPa (Hendrickx & Knorr, 2002) and often synergism between pressure and temperature is observed (Buckow & Heinz, 2008). By increasing the process pressure, it is possible to decrease the temperature needed to achieve the same inactivation. According to Smelt et al. (2001) the pressure induced effects leading to cell death of vegetative microorganisms can be attributed to four factors:

(I) Protein and enzyme unfolding, including partial or complete denaturation;
(II) Cell membranes undergoing a phase transition and change of fluidity;
(III) Disintegration of ribosomes in their subunits; and
(IV) Intracellular pH changes related to the inactivation of enzymes and membrane damage (Knorr et al., 2011; Molina-Gutierrez, Stippl, Delgado, Gänzle, & Vogel, 2002).

2.2. Sporulated microorganisms' inactivation by HPP

While high pressure pasteurization is already established, high pressure sterilization is not yet implemented within the food industry, even though the process of Pressure Assisted Thermal Sterilization for mashed potatoes filed at the FDA was accepted in 2009 (Illinois Institute of Technology, 2009). This lies mostly in the current limitation in achieving full spore inactivation and a gap of knowledge in the mechanistic impact of high pressure in combination with high temperature on spores. Moreover, the lack of alignment in the choice of a (product-specific) bacterial strain(s) for validation and the absence of industrial-scale systems with adequate temperature homogeneity at

final pressure/temperature further restrict the current adoption of this process by industry.

The lack of temperature uniformity during high pressure high temperature processing within industrial vessels has been reported by several research groups and can be explained by differences in adiabatic heating of the product, pressure medium and the heat transfer from the vessel wall (Grauwet et al., 2012; Juliano, Knoerzer, Fryer, & Versteeg, 2009; Knoerzer, Juliano, Gladman, Versteeg, & Fryer, 2007). The mapping of the temperature inhomogeneity has been described by a quantitative analysis of temperature using three-dimensional numerical simulations (Rauh, Baars, & Delgado, 2009) or by the use of a pressure temperature time indicator, a protein-based indicator which can be placed on several positions within the vessel and the read-out can be conducted after the treatment (Grauwet, Plancken, Vervoort, Hendrickx, & Loey, 2010). This absence of temperature homogeneity at high pressure represents to date the main technological limitation to the implementation of high pressure thermal sterilization in industry. "Computational Fluid Dynamics" modeling was suggested as a mean to design carriers which produce thermal uniformity within the carrier (Knoerzer, Buckow, Chapman, Juliano, & Versteeg, 2010; Knoerzer et al., 2007). Knoerzer et al. (2007) established a model predicting the flow and temperature fields during a sterilization process in a pilot scale (35 L) high pressure high temperature vessel without carrier, with metal composite carrier and with a polytetrafluoroethylene (PTFE) carrier. Their model represented well the experimental data and temperature uniformity was reached with a PTFE carrier, acting as a barrier for flow and heat transfer. Simulation of a high pressure sterilization process at 600 MPa and ~121 °C showed that more than 94.6% of the PTFE carrier volume achieved a 12 $\log_{10}$ reduction or more based on the F-value concept ($z = 10$ °C, $T_{ref} = 121.1$ °C and $D_{121.1\ °C} = 0.21$ min for *Clostridium botulinum* spores). However, only thermal inactivation was considered in this model and this approach neglects a possible effect of pressure as an additional hurdle for inactivation. It would be interesting for future models to include pressure and temperature dependent inactivation models.

From the microbial indicator perspective, *Clostridium* spores (*C. botulinum*, *Clostridium sporogenes* and *Clostridium perfringens*), *Bacillus* spores (*Bacillus amyloliquefaciens*) and *Geobacillus stearothermophilus* spores are mentioned by numerous research groups as being very highly pressure and temperature resistant (Ahn, Lee, & Balasubramaniam, 2014; Juliano et al., 2009; Margosch et al., 2006; Rajan, Ahn, Balasubramaniam, & Yousef, 2006; Wimalaratne & Farid, 2008). One of the main difficulties in selecting a reference indicator strain is that some of the known target microorganisms for thermal sterilization are high pressure sensitive and that some high temperature sensitive microorganisms can become very resistant under high pressure conditions (Margosch et al., 2006; Sevenich et al., 2013). In addition, matrix specific effects might impact on inactivation and it has been suggested that future validation scheme for high pressure high temperature sterilization should be based on product-specific, relevant, indicator strains (Eisenbrand, 2005).

The inactivation mechanisms of bacterial endospores by high pressure, at least partly differ from the ones described for vegetative microorganisms. This is mainly attributed to the inactive metabolism, the presence of multiple protective layers, small acid soluble proteins protecting the DNA and the low water content in spores. It is widely accepted that the inactivation of spores under high pressure is at least a two-step inactivation (Heinz & Knorr, 2002; Margosch, Gänzle, Ehrmann, & Vogel, 2004; Mathys, Chapman, Bull, Heinz, & Knorr, 2007; Reineke et al., 2011; Wuytack, Boven, & Michiels, 1998). Most of existing mechanistic studies only focus on the first step, which is the release of dipicolinic acid and accompanied by a loss of heat resistance (Black et al., 2005; Gould & Sale, 1970; Paidhungat et al., 2002; Wuytack, Soons, Poschet, & Michiels, 2000; Wuytack et al., 1998), or on the last step, the inactivation (Ardia, 2004; Margosch et al., 2006; Mathys, Reineke, Heinz, & Knorr, 2009). Only some studies have measured and discussed the entire mechanism (Heinz & Knorr, 1996; Margosch et al., 2004; Mathys et al., 2007) among which, a study covering these mechanisms and a broad pressure–temperature and time range, was presented by Reineke (2012) (Fig. 1). Some recent studies opened new approaches to investigate spore inactivation mechanisms by focusing on the in situ investigation of spore high pressure germination and inactivation (Georget et al., 2014; Hofstetter, Winter, McMullen, & Gänzle, 2013). In situ investigations might be a valuable tool to fill the gaps in our understanding of spore inactivation mechanisms.

Three different pressure temperature domains were proposed, for the inactivation of *Bacillus subtilis* spores in ACES-buffer solution. The first domain (from 0.1-600 MPa and 30-50 °C) covers the non-nutrient pressure induced spore germination, which only leads to spore inactivation (max. 4 $\log_{10}$) after long pressure dwell times (>1 h) (Reineke, Mathys, Heinz, & Knorr, 2013). In the second domain (from 0.1 to 600 MPa and temperatures < 60 °C) the combination of pressure and temperature directly affects the inner spore membrane and/or membrane channel proteins, leading to a spore core hydration and a subsequent inactivation, which is the fastest in domain three (pressure > 600 MPa and temperatures > 60 °C), presumably due to a full loss of barrier properties of the inner spore membrane (Reineke et al., 2013). However, most of these inactivation mechanisms are derived in simple aqueous systems, such as buffer solutions and can be affected by food constituents. The food system itself could have a protective effect on the spores because certain ingredients such as fats, sugars, and salts can interact with bacterial spores in a protective way. The resulting a_w of the food could lead to retarded inactivation (Ababouch, Grimit, Eddafry, & Busta, 1995; Oxen & Knorr, 1993; Senhaji & Loncin, 1977; Sevenich et al., 2013) and spore inactivation needs to be tested in real food systems to ensure the safety of this process.

Although not adopted by the food industry, two sterilization concepts have been investigated. Pressure Assisted Thermal Sterilization (PATS) (Illinois Institute of Technology, 2009) only considers the thermal sterilization temperature being reached during pressure dwell time as the lethal factor whereas High Pressure Thermal Sterilization (HPTS) also takes into account the impact of pressure on spore inactivation (Mathys et al., 2009). In both cases, the process relies on adiabatic heating to bring the product to sterilization temperatures. For HPTS treatments, two approaches have been suggested. The first one consists of a single pressure pulse (Heinz, 1997) while the second one consists of multiple pulses (Meyer, 2000). However, multiple cycles reduce the lifetime of high pressure equipment and increase maintenance costs. This second approach is thus not recommended for technical and economic reasons (Reineke, Mathys, & Knorr, 2011a).

During compression, the work of compression leads to temperature changes. The theoretically resulting adiabatic heating can be derived from the first law of thermodynamics (Bridgman, 1912) and leads to the maximal achievable temperature change. In uniform material the temperature rise happens simultaneously in the whole product. The extent of the temperature rise depends on the material properties (adiabatic heating depending on the compressibility and the specific heat of the substance) and overlapping heat transfer phenomena (non-adiabatic conditions). Different pressure transmitting media would result in a variable adiabatic heating (Ardia, 2004). However, due to the lack of thermophysical data on real foods under pressure, the modeling of adiabatic heating in real system has been limited (Ardia, Knorr, & Heinz, 2004). Mainly practical measurements were able to demonstrate the differences of the adiabatic heating in real food systems. The relevant knowledge of adiabatic heating during HPP of foodstuff is summarized in Table 1 (Gupta & Balasubramaniam, 2012; Ting, Balasubramaniam, & Raghubeer, 2002). The main ingredient in most food is water and thus the thermophysical properties of water have been utilized to estimate the temperature increase upon compression of high moisture foods. However, when working with fatty/oily matrices or emulsions, this approximation is not accurate. As shown in Table 1, the compression heating in fat containing foods could be up

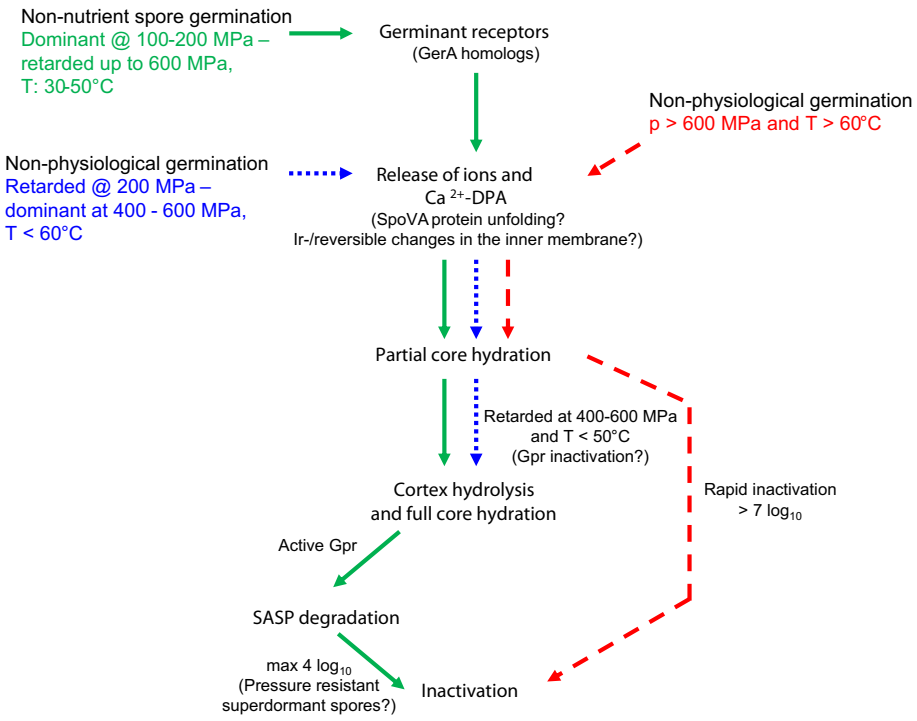

Fig. 1. Proposed germination and inactivation pathways of *Bacillus subtilis* in dependence of the applied pressure temperature conditions. Reprinted from Reineke et al. (2013), with permission from Elsevier.

to three times higher than for water (Ting et al., 2002), highlighting a potential application for HPTS or PATS. The heat transfer from the pressure transmitting medium into the product could be used to increase the temperature of the food system during and after the adiabatic heating. It would thus achieve much faster lethal conditions (Toepfl, Mathys, Heinz, & Knorr, 2006), in which case, however, non-uniform temperature distributions would need to be accounted for. Nevertheless, results of Sevenich et al. (2013) showed for the food systems tuna in sunflower oil and sardine in olive oil that the protective effect of the oil and a retardation of the spore inactivation were still present at high temperatures (110–115 °C) although the target temperature was reached faster by adiabatic heating. This could be explained by a low a_w of the foods in a range of 0.91–0.93. Further investigation and potential modeling of adiabatic heating of fat containing as well as emulsified foodstuff would be required to achieve controlled heating of these challenging matrices. It is furthermore important to consider that oil systems exhibit nonlinear adiabatic heating with increasing pressures. Finally, adiabatic heating of oils is independent from the initial temperature, which differs from water based systems. The impact of fat in food matrices is further discussed in Section 3.3.

2.3. Modeling of inactivation kinetics by HPP

While this is not the core focus of this review, it remains important to address the topic of the microbial inactivation kinetics by HPP. In food microbiology, models of inactivation kinetics are commonly used to describe the inactivation of vegetative and sporulated microbial populations. When considering food preservation, it is essential to be able to exactly quantify the surviving microorganisms in order to maintain consumer's safety. In this regard, inactivation kinetic modeling takes all its importance. Different modeling approaches have been suggested and they can mainly be summarized in two subgroups: the mechanistic and vitalistic models (Lee & Gilbert, 1918; Watson, 1908).

The fundament of the original mechanistic conception is that the microorganism's inactivation is similar to a chemical reaction and time dependent (Lee & Gilbert, 1918). This conception led to the most common expression of microbial inactivation following a first order inactivation kinetic where the number of surviving microorganisms is logarithmically plotted versus time and the exponential inactivation curve becomes a linear function with the rate constant k as slope. This model is the basis of thermal processing evaluation (e.g. canning) for

Table 1
Heat of compression values of selected foods determined at initial sample temperature of 25 °C.
Adapted from Gupta & Balasubramaniam (2012), with permission from Elsevier.

Food sample	Temperature increase (°C) per 100 MPa
Water	3
Orange juice, tomato salsa, 2% fat milk, and other water-like substances	Ranging from 2.6 to 3.0
Linolenic acid	9.0–5.9[a]
Soybean oil	9.1–6.2[a]
Olive oil	8.7–6.3[a]
Crude beef fat	4.4
Extracted beef fat	8.3–6.3[a]
Beef ground	3.2
Gravy beef	3.0
Chicken fat	4.5
Chicken breast	3.1
Salmon	3.0
Egg albumin	3.0
Egg yolk	4.4
Egg whole	3.3
Mayonnaise	7.2–5.3[a]
Whole milk	3.2
Tofu	3.1
Mashed potato	3.0
Yoghurt	3.1
Cream cheese	4.8
Hass avocado	4.1–3.7[a]
Honey	3.2
Water/glycol (50/50)	4.8–3.7[a]
Propylene glycol	5.3
Ethanol	10.6–6.8[a]

[a] Substances exhibited decreasing adiabatic heating as pressure increased (Patazca, Koutchma, & Balasubramaniam, 2007; Rasanayagam et al., 2003; Ting et al., 2002).

treatments with variable temperatures and has led to the 12 D-concept, still commonly used in the industry (Kessler, 2002). However, when considering high pressure inactivation of microorganisms, with or without combination with high temperature, inactivation kinetics frequently do not follow a first order inactivation kinetic (Cheftel, 1995; Heinz & Knorr, 1996; Heinz & Knorr, 1998; Metrick, Hoover, & Farkas, 1989). In particular, an initial lag phase ("shoulder") and/or a leveling off for extended treatment times ("tailing") are common deviations to the log linear microbial inactivation. In order to model nonlinear inactivation curves, multiple primary models have been proposed. Using an nth-order model instead of a first order model, tailing behaviors could be modeled but not shoulder (Van Boekel, 2010). This was shown practically for high pressure high temperature spore inactivation for instance by Reineke (2012). Moreover, the n parameter has no mechanistic background (Van Boekel, 2010).

Vitalistic models are based on the hypothesis that all individual microorganisms are different in their response to stresses such as temperature and pressure for instance (Lee & Gilbert, 1918) and that consequently the survival curve represents a distribution of lethal events in time. These models account well for the shoulder and tailing behaviors frequently observed and several suggestions can be found in the literature (Peleg & Cole, 1998; Van Boekel, 2010). In particular, Weibull model, derived from reliability engineering, allows for a great fitting of microbial inactivation curves and an adaptation such as the double Weibullian model has been applied to non-log linear inactivation of vegetative and sporulated microorganisms. Other empirical models such as the modified Baranyi model were also suggested to describe accurately nonlinear inactivation curves (Koseki & Yamamoto, 2007b). The Weibull model was assessed by Reineke, Doehner, et al. (2011) to model isorate lines for the high pressure inactivation of *B. subtilis* spores. In spite of a good individual fit of each kinetic, no systematic pressure and temperature variation of the model parameters could be found, thus limiting the application of these models to complex inactivation phenomenon or for predictive applications. In this case, the inactivation pressure temperature isorate lines could not be obtained. Instead, Reineke, Doehner, et al. (2011) showed that a multiresponse kinetic modeling based on the combination of simple chemical differential equations linking the different state of the bacterial spores (dormant, germinated, and inactivated) could also offer a mechanistic based satisfactory modeling of inactivation curves. Alternatively, Heinz and Knorr (1996) showed that a combination of the Weibull distribution and a mechanistic model (1st order kinetic) in a two-step-model considering distributed resistance mechanisms could be applied to model the high pressure inactivation of cells of *B. subtilis*. Most interestingly, in this case regressively derived parameters showed a log-linear behavior as function of pressure which supports the capacity of this model to represent vegetative microorganism's high pressure inactivation.

For a detailed overview of these various modeling approaches, the reader is referred to the corresponding literature cited in this section.

2.4. Impact of pK_a and dissociation equilibrium shift on high pressure inactivation

Water is vital to all known forms of life and is the major component of most food systems as well as microorganisms. Consequently, pressure induced changes in aqueous systems are important with regard to inactivation mechanisms of microorganisms.

One of the most important changes of aqueous systems under pressure is the shift of the dissociation equilibrium, observed both in water and in simple chemical systems such as buffer solutions or cell cytoplasm. The thermodynamic background of a dissociation reaction in diluted solutions is shown in Eq. (1).

$$HA + H_2O \leftrightarrow A^- + H_3O^+ \quad (1)$$

where HA is an acid, H_2O is water, A^- is the conjugated base, and H_3O^+ is the oxonium ion. The concentration of water remains basically unaffected during the dissociation of a base or an acid, since it is large (55.5 M) by comparison to the other concentrations. It can thus be omitted in the law of mass action. Consequently, the acid dissociation constant K_a can be calculated with Eq. (2)

$$K_a = \frac{\gamma_{H_3O^+} \cdot \gamma_{A^-}}{\gamma_{HA}} \cdot \frac{[A^-] \cdot [H_3O^+]}{[HA]} \quad 2$$

with the activity coefficients γ_i. The acid dissociation constant K_a characterizes the extent of the dissociation of oxonium ions from an acid. This constant differs for each acid and fluctuates over many degrees of magnitude, therefore the acidity constant is often expressed by the additive inverse of its common logarithm (Degner, 2009), define as pK_a (Eq. (3)).

$$pK_a = -\log_{10}(K_a) \quad 3$$

The activity of oxonium ions, which can also vary over an extensive range, is essential to describe the thermodynamic and kinetic properties of processes which occur in aqueous solutions and is commonly expressed through the pH (Eq. (4)).

$$pH = -\log_{10}\left(\gamma_{H_3O^+} \cdot \frac{[H_3O^+]}{1\ mol/L}\right) \quad 4$$

The pH value would be changed with all reaction partners during the shift of dissociation equilibria, (Eq. (1)), but no change of the concentration difference on one site happens during the reaction. For instance, in water at 0.1 MPa and 20 °C with nearly equal oxonium ($[H_3O^+]$) and hydroxide ($[OH^-]$) concentrations, neutral conditions ($[H_3O^+] = [OH^-]$) are present. Oxonium and hydroxide concentrations increase under pressure, but "neutral" conditions remain. It results that the pH shift alone cannot accurately characterize the dissociation equilibrium shift for aqueous systems under high pressure, and for that reason the pK_a shift should be used (Mathys, Kallmeyer, Heinz, & Knorr, 2008).

The dissociation equilibrium is pressure and temperature dependent, which can markedly influence thermal processing, such as pasteurization, sterilization or freezing as well as preservation processes under high pressure. For basic inactivation investigations of microorganisms under high pressure, buffer solutions are ordinarily used to achieve constant pH-values and medium properties. However, even in such simple model systems, the pressure induced dissociation equilibrium shift could have an impact and cause a bias in the results of inactivation experiments (Mathys et al., 2008; Reineke, Mathys, & Knorr, 2011b). This was first confirmed by Clouston and Wills (1969), who found different logarithmic reduction of *Bacillus licheniformis* spores in water and phosphate buffer after low pressure inactivation up to 170 MPa. The effect of a varying dissociation equilibrium is also important for high pressure-treated food, because the pK_a shift, pressure and temperature can act synergistically on the inactivation of microorganisms.

To quantify the variations of the pK_a value under pressure Distèche (1959) developed one of the first pressure-resistant pH-glass electrodes, used to measure the pH-value of sea water in the Mariana Trench at 110 MPa. To date, the dissociation equilibrium shift under pressure cannot be measured in solid foods, but a few optical measurement methods have been developed for liquids in situ pH determination. They can operate at pressures up to 250 MPa (Hayert, Perrier-Cornet, & Gervais, 1999; Quinlan & Reinhart, 2005) and 450 MPa (Stippl, Delgado, & Becker, 2002, 2004). However, these experimental methods are still limited and not appropriate for the study of combined thermal and pressure effects in complex matrices. Data about the pure impact of the pressure induced dissociation equilibrium shift on the inactivation of microorganisms are rare and cannot be isolated from the effects of other

food ingredients, which will be discussed in the next section. Nonetheless, the dissociation equilibrium shift under pressure should not be neglected in order to avoid potential errors in inactivation data interpretation.

3. High pressure inactivation of microorganisms in food matrices with low or variable water activity

3.1. Impact of different solutes and resulting microorganism protection against HPP inactivation

Water activity as food matrix parameter was introduced in the 1950s and is a measure of the amount of "free" or unbound water present in the food. It was defined as the ratio of the vapor pressure of water in a given matrix to the vapor pressure of pure water at the same temperature. It should not be directly compared with the water content (g water/g sample) which is the sum of the bound and unbound water. The a_w, ranges from 0 (absolute dryness) to 1 (free water). It accounts for water that can take an active part in exchange with the ambient humidity and can possibly be used for microbiological growth. Classically, no bacterial growth is expected for an a_w inferior to 0.90 (Barbosa-Cánovas & Vega-Mercado, 1996) however some halotolerant bacteria such as *Staphylococcus aureus* can grow at a_w as low as 0.82 (Grant, 2004). This emphasizes the need to combine low a_w with additional hurdles in order to guarantee food safety. The survival of microorganisms under such low a_w-conditions is based on their ability to gain or regain turgor pressure. Once bacteria are exposed to low a_w the internal turgor pressure can be lost and the cell becomes flaccid or plasmolyzed (Sperber, 1983).

One mean to reduce the a_w is by increasing the concentration of solutes and it is well known that the resulting bactericidal effect of temperature is reduced when applied to solutions with high osmolarity (Gaillard, Leguerinel, & Mafart, 1998; Kessler, 2002; Murrell & Scott, 1966). The same effect was observed during HPP inactivation (Cheftel, 1995). Several attempts have been made to use the a_w to generalize the results on the effect of the solute concentration on HPP induced inactivation of microorganisms (Hayman, Kouassi, Anantheswaran, Floros, & Knabel, 2008; Koseki & Yamamoto, 2007a; Molina-Gutierrez, Rademacher, Gänzle, & Vogel, 2002; Molina-Höppner, Doster, Vogel, & Gänzle, 2004; Moussa, Perrier-Cornet, & Gervais, 2006; Oxen & Knorr, 1993; Simpson & Gilmour, 1997). Early investigation looked at the physiological impact of low a_w environment on vegetative microorganisms. Some bacteria and fungi showed the ability to regain their internal turgor pressure and adapt to the new low a_w environment by an osmoregulatory process. This includes an initial sensing step, a translation step in which a response is triggered followed by a physiological accommodation step and eventually growth (Troller, 1987). This adjusting mechanism conducted by yeasts (Brown, 1978) and osmotolerant bacteria (Gould, Measures, Wilkie, & Meares, 1977) is accompanied by the production of "compatible compounds" to adjust to the low a_w (Fig. 2).

The uptake of K^+ in the vegetative cell plays a key role in the deplasmolysis and is coupled with a complex intracellular transport system. K^+ is needed in the cell to keep the balance of charge within the cell if exposed to an environment with low a_w. The high concentration of K^+ promotes the activity of certain enzymes, mostly glutamate dehydrogenase which leads to the conversion of α-ketoglutarate into glutamic acid. Glutamic acid reduces the intracellular a_w which leads to the uptake of water and therefore brings the cell back to a balanced state and an osmotic initial equilibrium. Some microorganisms further convert the glutamic acid to proline or γ-aminobutyric and thus avoid the energy intensive maintenance of the K^+-uptake (Lenovich, 1987). These compounds are also able to reduce the internal a_w and attract water to restore the initial state of cell and are called compatible solutes since they do not interact or interfere with the metabolic pathways of the cell. How this high concentration of solute and the final a_w alters inactivation by high pressure was the object of later work. Early investigations on this topic were reported by Oxen and Knorr (1993) on the inactivation of the yeast *Rhodotorula rubra* and demonstrated the different degrees of baroprotective effect at 400 MPa, 25 °C and 15 min among solutions of glucose, sucrose, fructose or sodium chloride and for equivalent a_w. This work showed that for $a_w < 0.92$ inactivation was inhibited regardless of the solute. However, at $a_w = 0.94$, a clear impact of the type of solute was visible in agreement with later work by Koseki and Yamamoto (2007a). Similar results showed that a reduction of the a_w from 0.98 to 0.95 by means of increased sucrose concentration in broth conferred partial baroprotection to *Zygosaccharomyces bailii* for pressures up to 517 MPa (Palou, López-Malo, Barbosa-Cánovas, Welti-Chanes, & Swanson, 1997). This was in agreement with the work of Van Opstal, Vanmuysen, and Michiels (2003) on high pressure inactivation of *Escherichia coli* in presence of sucrose for pressures between 250 and 550 MPa. Interestingly, Van Opstal et al. (2003) additionally noticed that increased concentrations in sucrose (and respectively low a_w) were in turn hindering posttreatment recovery from sub-lethal damages to *E. coli*, leading to further inactivation up to 24 h following HPP, even in cases where no inactivation had been observed immediately after treatment. As a result, the net effect of a_w may be difficult to predict. However, this last observation also suggested a possible opening to improve microbial inactivation in low a_w matrices by HPP. Molina-Gutierrez, Rademacher, Gänzle, and Vogel (2002) and Molina-Höppner et al. (2004) reported the influence of sucrose or sodium chloride on the HPP-induced inactivation of *Lactococcus lactis* for pressures between 200 and 600 MPa. It was argued that ionic and non-ionic solutes have a different mechanism of protection against high pressure mediated microbial inactivation. It was observed that sucrose protected the metabolic activity and the membrane integrity of *L. lactis* while salt did not show any protection on metabolic activity. Molina-Höppner et al. (2004) proposed that disaccharides act against pressure induced inactivation of cellular components while ionic solutes protection would only rely on the intracellular accumulation of compatible solutes as a response to the osmotic stress. This would offer an asymmetric protection against HPP and require higher concentrations (4.0 M NaCl) than disaccharides (0.5 M sucrose) to be effective. It might also be linked to the potential of sugars to interact with the cell membrane and cause a retardation of the shift from the liquid phase to the gel phase under high pressure. Molina-Höppner et al. (2004) concluded that the accumulation of disaccharides or compatible solutes could protect bacteria against pressure-mediated cell death in high concentration sodium chloride or sucrose solutions.

Koseki and Yamamoto (2007a) investigated the effect of a_w and saturation of solutions on HPP induced inactivation of *Listeria monocytogenes*, using three species in solution (sodium chloride: 0.2–5.0 M, sucrose: 0.9–2.0 M, and phosphate buffer: 0.01–1 M). The results of this study showed no consistent correlation between a_w and solute concentration in terms of the baroprotective effect. It was proposed that, at low a_w, proteins are inflexible and hence not denatured by HP-treatment in agreement with previous work (Hayman et al., 2008; Moussa et al., 2006). However, this hypothesis could not clarify why phosphate buffer (1.0 M, a_w ~ 0.96) fully inhibited HPP-induced inactivation at 400 MPa up to 10 min. Koseki and Yamamoto (2007a) observed that, as the saturation of suspensions increased, the effect of HPP-induced inactivation of *L. monocytogenes* decreased, regardless of the kind of solute. This suggested that the saturation of a solution might be a more reliable parameter to consider than a_w to explain the variable impact of different solutes at equivalent a_w. They proposed that when a solution is over saturated, the pressure transmission to the water might be inhibited. However, Hayman et al. (2008) found minimal inactivation of *L. monocytogenes* lyophilized and suspended in 100% glycerol and showed that the absence of inactivation was not linked to lack of direct contact with a pressure-transmitting fluid contrary to the hypothesis of Koseki and Yamamoto (2007a). Glycerol, a permeant solute, enters the cell by simple diffusion (Gould, 1985) and it has been known to have a protective effect on cells and proteins. It also affects the dynamic and thermodynamic properties of protein

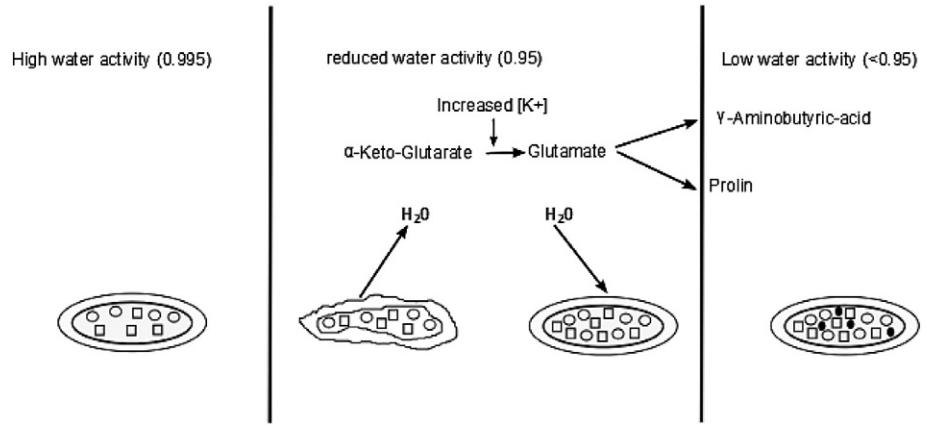

Fig. 2. Schematic drawing of the intracellular adaptation to low a_w: glutamate (○), potassium ions (□) and proline or γ-aminobutyric acid (●). Adapted from Sperber (1983).

solutions (Priev, Almagor, Yedgar, & Gavish, 1996; Zancan & Sola-Penna, 2005). Suspension of a protein in glycerol resulted in the displacement of water from the core of the protein, and hence decreased the volume and compressibility of the protein interior (Priev et al., 1996) and thus stabilized proteins against denaturation (Cioni & Strambini, 1994; Di Primo, Deprez, Hoa, & Douzou, 1995; Ruan et al., 2003; Zancan & Sola-Penna, 2005). The Koseki and Yamamoto (2007a) hypothesis was further challenged by the first systematic examination of the effect of buffer salt properties and concentration on inactivation of *E. coli* and *S. aureus* by HPP between 350 and 500 MPa (Gayán, Condón, Álvarez, Nabakabaya, & Mackey, 2013). The results in Table 2 shows that some salts were only protective at high concentration, relatively close to the saturation concentration (e.g. HEPES, KCl and NaCl), thus partially confirming the results of Koseki and Yamamoto (2007a). Gayán et al. (2013) argued that since concentrated solutions of salts are less compressible than pure water (Eder & Delgado, 2007; Min, Sastry, & Balasubramaniam, 2010) the reduction in the bulk compressibility of water might minimize the deformation of cell structures under pressure. However, the explanation for the baroprotective role of the other salts (e.g. Na_2SO_4 or $CaCl_2$) could not be correlated to the saturation of the solution and it was suggested that ion specific effects might be responsible. Both phosphate and sulfate anions are considered to be kosmotropes. Kosmotropes cause water molecules to favorably interact, which also stabilizes intermolecular interactions in macromolecules such as proteins, lipid bilayers and biological membranes (Zhang & Cremer, 2006). Calcium chloride (non-kosmotropic substance) protection was associated with the protective effect of calcium as stabilizer of pressure sensitive targets within the cell. When considering the specific impact of NaCl on vegetative cells inactivation by HPP, it would also be interesting to investigate whether NaCl exhibits antimicrobial properties besides its impact on a_w. This latter question is not the core objective of this work but it should be underlined that no consensus was yet reached in the scientific community with regard to the (absence of) toxicity of different ions in solution. Recent research showed that the interaction of HPP (up to 500 MPa at 20 °C for 6 min) with NaCl (0%–3%) resulted in lower microbial counts of the endogenous flora (below 2 log CFU/g) at the end of a 12 day storage of raw pork meat, than when HPP or NaCl alone was used (Duranton, Guillou, Simonin, Cheret, & de Lamballerie, 2012). However, the exact nature of the antimicrobial action of NaCl besides its impact on a_w and plasmolysis in non-halotolerant strains remains unclear and contradicting evidences in the literature underlined the need for additional research on this particular aspect as well as potential benefit in combination with HPP. To date, the main conclusion for most studies remains that NaCl exhibits a baroprotective effect during HPP and an inhibitory effect in the recovery medium (Taormina, 2010).

For bacterial spore inactivation in solutions with different concentrations of solutes there is comparatively little data available to date. The data presented by Gayán et al. (2013) regarding the impact of HPP on different buffer salts and the dissociation equilibrium shift in water is in agreement with the work of Mathys et al. (2008) (Table 2). Mathys et al. (2008) showed stronger *G. stearothermophilus* ATCC 7953 spore inactivation at high pressure (500–900 MPa) — high temperature (up to 80 °C), in buffers with negative reaction volumes (phosphate buffer PBS, $\Delta V = -22.8$ cm^3/mol) than in buffers with a positive ΔV (ACES, $\Delta V = +4.0$ cm^3/mol), due to the corresponding dissociation equilibrium shift and charge formation under pressure. However, in the work of Mathys et al. (2008), this observation was validated only for spore inactivation at lower buffer salt concentrations. No investigations on spore inactivation were conducted at the high concentrations investigated above for vegetative microorganisms. It is thus difficult to assess whether the observations of Gayán et al. (2013), made on vegetative microorganism baroprotection by high buffer concentrations, might be transferred to bacterial spores and baroprotection. *G. stearothermophilus* IFO 12550 spore inactivation by HPP was also investigated in the presence of glucose (6–12%), NaCl (3–6%) and ethanol (10–20%) (Furukawa & Hayakawa, 2000). There, a systematic reduction in inactivation rate under pressure of 60 MPa, 95 °C and up to 500 min was observed, but full inactivation was always achieved, probably due to a too small solute concentration to grant full baroprotection through a_w reduction in the conditions tested. Recent work by Sevenich, Thieme, Hecht, Rauh, and Knorr (2014) on *Bacillus amyloliquefaciens* spores showed that by using higher concentration of NaCl (1.2–2.7 mol/L) and sucrose solutions (0.83–1.7 mol/L) to control the a_w in the range [0.9–1], significant baroprotection was achieved during processing at 600 MPa/105 °C or 600 MPa/115 °C.

Table 2
Concentration of solutions having a baroprotective effect on *E. coli* and *S. aureus* in relation to the concentration of a saturated solution (reproduced from Gayán et al., 2013 with permission from American Society for Microbiology). All solutions at pH 7.0 and maximal temperature of 30 °C reached during HPP.

Substance	Concentration needed for maximum protection (M)	Saturating concentration (M)	% of saturation at concentration giving maximum protection
Tris	2.0	4.13	48
HEPES	2.0	2.25	89
MOPS	2.0	4.7	43
KCl	4.0	4.61	87
NaCl	4.0	6.13	65
Na_2HPO_4	0.061[a]	0.36	27
Dimethyl glutarate	0.1	0.27	37
Na_2SO_4	0.1	1.29	7.8
$CaCl_2$	0.1	6.71	1.5

[a] Based on the concentration of the disodium salt in 0.1 M buffer at pH 7.0.

Interestingly, at equivalent a_w, sucrose seemed to lead to an increased baroprotection by comparison to NaCl. This difference was however reduced at the higher processing temperatures and a_w. With sufficiently long holding times, inactivation could be achieved with a faster rate than by thermal processing alone.

The results of this section suggest that the mechanisms of HPP inactivation of microorganisms in presence of solutes are complex. There does not seem to be any simple correlation between the a_w and resistance to high pressure inactivation. Sugars (glucose, fructose and sucrose) and glycerol stabilized microorganisms considerably more against inactivation than other compounds and the mechanisms of baroprotection seem to be multiple. Different solutes and resulting microorganism protection against HPP inactivation have to be considered at two levels:

- The impact on the solution a_w, accompanied with a stabilization of proteins and membranes. This effect might be dominant in sporulated microorganisms, less likely to interact with solutes, or at very high concentrations.
- The individual solute properties which might provide an additional protection against HPP induced inactivation of microorganisms (e.g. kosmotropic solutes).

3.2. Impact of dehydration of food systems on the water activity and resulting microorganism protection against HPP inactivation

As described above, water content and a_w are distinct parameters. The water content alone is not sufficient to determine food safety or predict product shelf life. The relationship between water content and a_w is complex and related to the relative humidity and the water content. Typically, it is possible to have products with comparable water contents but different water activities. For instance, salami and cooked beef both have similar water contents (60%) but the a_w of salami is 0.82 and cooked beef is approximately 0.98. One can however link the reduction of a_w to the drying process in the sense that drying removes the free water from the system and contributes to reduce the water available for microbial growth, namely the a_w. For example, dried fruits have an a_w of 0.72–0.80, spices 0.58–0.72 (e.g. grinded pepper and cinnamon) or milk powder of 0.19 (Schmidt & Fontana, 2008). Dehydration has been practiced since ancient times to preserve food.

Dehydrated matrices are relevant to consider separately from other low a_w matrices since the absence or minimal presence of water as transmitting medium for high pressure represent a particular challenge to HPP preservation for this class of products. Additionally, some of these matrices are destined to be rehydrated (soups, milk powders) and/or used as ingredients (spices, nuts), thereby opening a risk for microbial growth through consumer usage. In its report on risk profile on pathogens and filth in spices, the FDA emphasizes the major outbreaks from microbial contaminants in spices between 1973 and 2010 as well as the type of pathogens found in spices (Van Doren, Dennis, Brandt, & Solomotis, 2013). The review identified fourteen spice-associated illness outbreaks occurring between 1973 and 2010 which resulted in 1946 reported human illnesses, 128 hospitalizations (7% of cases) and two deaths (0.1%). The readers are referred to the corresponding report where the summary of enteric illness outbreaks taking place during 1973–2010 associated with consumption of microbial contaminants in dried spices and seasonings or foods containing these contaminated ingredients is presented. Mostly incriminated were white and black peppers with *Salmonella* contamination. Aside from *Salmonella*, *B. subtilis* and *Bacillus cereus* related outbreaks were also reported. Moreover, a list of microbial pathogens detected in spices between 1985 and 2012 listed *Salmonella* spp., *Bacillus* spp., *C. perfringens*, *Cronobacter* spp., *Shigella* and *S. aureus* as the main pathogens detected in spices (Van Doren et al., 2013). While treatments such as ethylene oxide, steam treatment or irradiation are commonly applied to spices to reduce the risk of microbial contamination, these are not well accepted by consumers or not satisfactory from a quality aspect and alternatives are still sought for.

Literature concerning HPP of dehydrated matrices is scarce since the use of high pressure requires the presence of a transmitting medium in order to be efficient. Without water, most of the dry foods e.g. herbs and spices will come out of the HPP treatment as clumps. Butz, Heinisch, and Tauscher (1994) looked at the response of suspensions of spices and spice mixtures in water to high pressure treatment and samples were decontaminated after three pressure cycles (30 min at 80 MPa followed by 30 min at 350 MPa) at 70 °C at a minimum a_w of 0.91. This however required preliminary hydration of the spices and no inactivation was noticed below a a_w of 0.66. Similarly, raw almond surface decontamination from *Salmonella enteritidis* by HPP (up to 482 MPa and 55 °C) was reported where only direct suspension in water proved to bring significant inactivation (Goodridge, Willford, & Kalchayanand, 2006; Willford, Mendonca, & Goodridge, 2008). This higher lethality of immersed almonds was attributed to the absorption of moisture at the surface and leading to an increased a_w. Alternatively, high pressure processing of vacuumed packaged nuts (pecans and walnuts) was assessed for inactivation of *Salmonella* but was reported to induce less than 1 $\log_{10}$ reduction at 600 MPa up to 20 min (Harris, 2013).

Further studies focused on the use of high pressure for decontamination of seeds. Neetoo, Ye, and Chen (2008) and Peñas, Gómez, Frías, and Vidal-Valverde (2008) used high pressure for the decontamination of mung bean and alfalfa seeds. In the case of Neetoo et al. (2008) the seeds were inoculated with the *E. coli* O157:H7 and successful inactivation could only be achieved after wetting of the seeds with a minimal volume (Neetoo, Pizzolato, & Chen, 2009; Neetoo et al., 2008). Dry treatment only induced minor inactivation as could be expected from the absence of pressure transferring medium. Similarly, Peñas et al. (2008) also wetted the beans prior treatment and vacuumed packed them (Peñas et al., 2008). In terms of food safety, sprouted seeds are a unique challenge since they offer optimal conditions of moisture, temperature and nutrients. In this case, high pressure processing could be an alternative for the decontamination (Neetoo et al., 2008). With high pressure, the safety of sprouts could be improved and one might prevent such EHEC outbreaks as in Germany in 2011, where contaminated sprout seeds led to a fatal outbreak (Robert Koch Institute, 2011).

Finally, dry meat products, though challenging due to the additional presence of high salt concentrations and low a_w might also beneficiate from HPP. For this product group, HPP might be a powerful tool to control risks associated with *Salmonella spp.* and *L. monocytogenes* as well as spoilage microorganisms in raw or marinated meats. For example, dry cured ham is a dry, bone-in, salted and dried, and non-fermented meat product. Due to its low a_w and high salt content, spoilage microorganisms, mainly gram-positive cocci and yeasts, might prove challenging to inactivate (Hugas, Garriga, & Monfort, 2002). Slicing of dry cured ham can lead to contamination of the final sliced products with spoilage or pathogenic flora such as *L. monocytogenes*. In the work of Garriga, Grèbol, Aymerich, Monfort, and Hugas (2004), sliced, skin vacuum-packed dry cured ham samples (a_w 0.89, NaCl 4.60%), were treated by HPP at 600 MPa for 6 min and showed at least 2 $\log_{10}$ reduction for spoilage associated microorganisms after treatment. *L. monocytogenes* was present (in 25 g) in only one untreated sample, at time 0, but absent in all HPP treated samples during the whole 4 °C storage period investigated (120 days). Furthermore, later work showed that 600 MPa for 6 min at 31 °C permitted to reduce a cocktail of inoculated *Salmonella* strains from 3.5 $\log_{10}$ CFU/g to <10 CFU/g dry-cured ham (Jofré, Aymerich, Grèbol, & Garriga, 2009). As mentioned in the previous section, one key consideration stated throughout studies on HPP preservation of dry-cured meat products is that, though limited during HPP processing, microorganism inactivation is reinforced post-processing due to the low a_w of the product treated which prevents the recovery of cells with sub-lethal damages. These findings were successfully translated into an industrial application by the company

Espuña, SA in Spain, which uses HPP to decontaminate packed cured ham (Espuña SA, 2014).

From these studies, it can be concluded that HPP of dried systems has limited application in particular due to the absence of a pressure transmitting continuous phase. Moreover, even under vacuumed conditions and using water as compression medium, the low surface a_w (for nuts for instance) still limits strongly the resulting microbial inactivation. Wetting and HPP of some dry systems such as seeds may nonetheless offer an interesting solution where no alternative has been this far established to fill the safety gap. In the case of sprouted seeds, one could possibly imagine a HPP step preceding the sprouting step to reduce the final microbial loads.

Dry-cured meat products are one of the most promising applications of HPP for the dry product category as was shown with the industrial application by Espuña, SA in Spain, now commercialized across Europe. HPP showed potential to reduce spoilage and pathogenic flora. Though small, these reductions might be significant when considered in a product with low initial contamination by *Listeria* spp.

3.3. Impact of fats and oils on local water activity in food matrices and resulting effect on microbial inactivation under HPP

Fats and oils are special cases of low a_w food matrices. In addition, fat containing emulsions, though containing free water, might create local low a_w refuges.

Cheese, with its high fat and salt content constitutes one of the matrices of interest for HPP inactivation. Cheese a_w can vary greatly depending on the type of cheese and processing and covers a range from 0.69 (e.g. parmesan) to 0.99 (e.g. cream cheese) (Schmidt & Fontana, 2008). The main microorganisms of interest in this product category are *L. monocytogenes*, *E. coli*, *S. aureus* and spoilage microorganisms. A number of studies have focused on HPP inactivation in diverse cheeses (Morales et al., 2006; Reps, Kolakowski, & Dajnowiec, 1998; Szczawinski, Szczawinski, Stanczack, Fonberg-Broczek, & Arabas, 1997; Voigt, Chevalier, Qian, & Kelly, 2010). Capellas, Mor-Mur, Gervilla, Yuste, and Guamis (2000) reported up to 4.9 $\log_{10}$ reduction of spores of *B. subtilis* in fresh cheese by using successively high pressure germination at 60 MPa/40 °C and high pressure inactivation of the germinated spores at 500 MPa/40 °C. Morales et al. (2006) found that the a_w of cheese significantly affected the pressure resistance of *L. monocytogenes* and suggested that fat increased the resistance of microorganisms to HPP destruction in a similar way as to heat inactivation.

Many natural and formulated dairy products are oil in water emulsions. Cream is an example which offers a challenging environment for microbial inactivation due to the presence of fat and corresponding locally reduced a_w. Early work nonetheless suggested that the microbial load of cream (35% fat) could be significantly reduced. *Listeria innocua* could be inactivated by HPP at 450 MPa and 25 °C for 10–30 min and it was suggested that HPP might help extending the refrigerated shelf life of dairy creams (Raffalli et al., 1994). Simpson and Gilmour (1997) later showed that high pressure inactivation of *L. monocytogenes* was significantly reduced when cells were suspended in an olive oil mixture (30% v/v oil) by comparison to a PBS buffer. The authors suggested that the reduced level of water in the fat droplets or at their interface might have been a determining factor in conferring baroprotection. As might be expected, at reduced fat content (e.g. milk at 3.5%), the protective effect of fat in emulsions also diminishes suggesting microorganisms might not remain in or at the interface of the fat droplets. For instance, fat content in milk between 0, 3.25 and 5% had no significant influence on the HP destruction of *E. coli* and it was further found that the major contributors for baroprotection of *E. coli* in milk during HP treatment appear to be casein and lactose, rather than the fat content (Ramaswamy, Jin, & Zhu, 2009).

The scarcity of studies investigating bacterial spore inactivation under HPP in fatty/oily or emulsified matrices has to be emphasized. Ananta, Heinz, Schlüter, and Knorr (2001) showed that by adjusting moisture content of cocoa mass to 30%, the inactivation of *G. stearothermophilus* spores was clearly pressure and temperature dependent and up to 6 $\log_{10}$ reduction was achieved. These observations showed that the spores were effectively protected by their surrounding matrix against lethal effects of pressure and heat. Spore inactivation was enhanced by the addition of water to the medium. This approach was first elucidated from heat inactivation studies of spores in fat systems and the variable protection conferred by different oils was associated with variations in their water content (Molin & Snygg, 1967). It was further argued that the protective effect of fat on the suspended microorganism was due to the decreasing a_w of the system, which possibly affected the spore germination system in such a way that the spores could not germinate and be inactivated (Furukawa & Hayakawa, 2000; Raso, Góngora-Nieto, Barbosa-Cánovas, & Swanson, 1998). The addition of water might have contributed to creating a more suitable environment, which allowed bacterial spores to germinate. Plausible mechanisms related to this behavior were suggested (Ananta et al., 2001). They ranged from shifting the specific solute driven osmotic condition into a more favorable one, increased solvation of spore components (Clouston & Wills, 1969), to migration of spores from fatty into aqueous regions or diffusion of water into fatty regions (Senhaji & Loncin, 1977). However, the migration of spores into aqueous regions seems unlikely due to the hydrophobic nature of bacterial spores' surface (Wiencek, Klapes, & Foegeding, 1990).

Inactivation kinetics of *B. amyloliquefaciens* spores in fish in oils, were reported and also highlighted a protective role of the matrix probably linked to the reduced a_w associated with the presence of oil. This latter study focused on the use of HPTS in different food and model systems with varying a_w including fish in different oils, in brine and baby food (Sevenich et al., 2013; Sevenich et al., 2014; Sevenich et al., 2015). The authors focused on the inactivation of two selected spore strains, *B. amyloliquefaciens* (Technische Mikrobiologie Weihenstephan, 2.479, Fad 82) and *G. stearothermophilus* (Sterikon with bioindicator), at different temperature-time combinations (90–121 °C, 0–30 min) at 600 MPa (HPTS) and showed that spores of *B. amyloliquefaciens* were more resistant to HPTS than the ones of *G. stearothermophilus*. The T, t dependencies at 600 MPa in real and model systems at variable a_w (baby food puree a_w: 0.96, fish systems in oils or brine a_w: 0.91–0.94 and ACES buffer 0.05 M a_w: 1) were established for 5 $\log_{10}$ and extrapolated for 12 $\log_{10}$ inactivation and the corresponding inactivation kinetics modeled with a nth-order model (Fig. 3). Fig. 3A shows that an inactivation of 5 $\log_{10}$ for the tested spore strain is possible with HPTS even at relatively low temperatures (90 °C) although depending on the matrix, longer holding times are necessary to achieve the desired inactivation. For 5 $\log_{10}$ inactivation, the influence of the a_w in the ranges considered, was rather low at high temperatures. However, a baroprotective effect of the matrix was apparent for lower temperatures ($T \leq 110$ °C and $t \geq 10$ min).

In the canning industry the 12 D-concept is often applied to guarantee safe and stable foods. To evaluate under which condition HPTS could lead to a 12 $\log_{10}$ inactivation, an extrapolation was carried out for the isokinetic lines (Fig. 3B) and indicated that the a_w could have a protective effect on the spores. Kinetics in olive oil (dotted line) and tuna in sunflower oil (dashed line) with an a_w between 0.92 and 0.91, suggested that oil offered a protective effect. The baby food puree and tuna in brine with an a_w between 0.94 and 0.96 did not offer a protective effect and therefore lower T,t-combinations were possible.

Other authors, who investigated thermal inactivation of spores, suggested the existence of free fatty acids, which could enter inner parts of the spores and protect key molecules from heat denaturation. This conclusion was drawn following heat inactivation of spores suspended in various lipid materials with the same water level (Ababouch et al., 1995; Molin & Snygg, 1967). This hypothesis was however challenged by more recent work emphasizing a potential beneficial role of unsaturated fatty acids in the HPP inactivation of microorganisms in food matrices as described below.

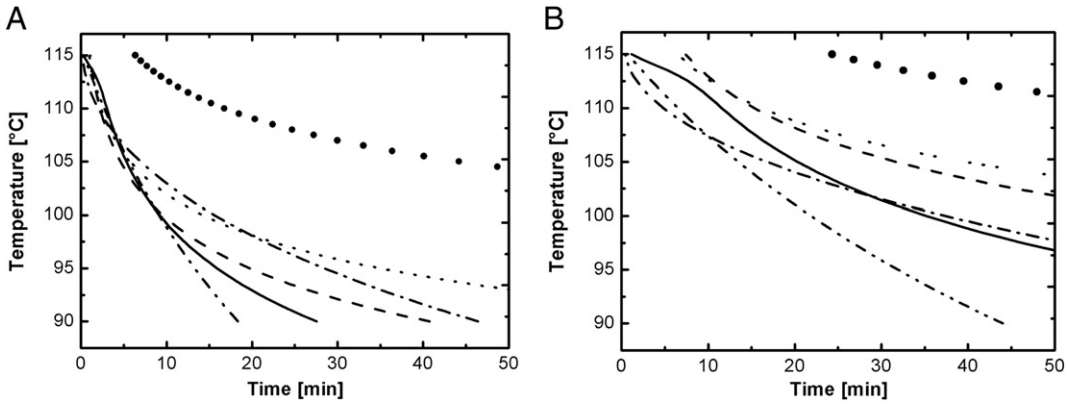

Fig. 3. Isokinetic lines of selected food systems for an 5 $\log_{10}$ (A) and extrapolated 12 $\log_{10}$ (B) inactivation of *B. amyloliquefaciens* spores at 600 MPa in a T,t-range of 90–115 °C and 0–50 min. Isokinetic lines were derived from kinetic analysis of the experimental inactivation data. Sardine in olive oil (small black dotted line — a_w 0.92 — n = 1.1); tuna in sunflower oil (black dashed line — a_w 0.91 — n = 1.1); ACES-buffer 0.05 M (solid black line — a_w 1 — n = 1.05); baby food puree (black dash dotted line — a_w 0.96 — n = 1); tuna in brine (dash dot dotted line — a_w 0.94 — n = 1); border of the thermal inactivation (large black dots) (based on Sevenich et al., 2013; Sevenich, Kleinstueck, et al., 2014; Sevenich, Thieme, Hecht, Rauh, & Knorr, 2014; Sevenich et al., 2015, with permission from Elsevier). For reaction rate constants, the reader is referred to the corresponding literature sources.

Tests conducted on fatty duck liver (44% fat) stabilized the livers for 90 days at 4 °C by means of a high pressure treatment (550 MPa/55 °C/20 min) and appropriate vacuum packaging. In this work, the indicators selected were *S. aureus*, the total coliforms and the total aerobic mesophilic flora. The final product presented improved sensory properties by comparison to the standard process, but no mechanism was presented (Ballestra et al., 1999; Cruz et al., 2003; El Moueffak et al., 1994; El Moueffak et al., 2001). Later work looked at the effects of HPP (500 MPa, 5 min) on the microbiological, physico-chemical and sensory properties of fermented Spanish dry sausages (salchichón), high in unsaturated fatty acids (high-oleic and high-linoleic acids) (Rubio, Martínez, García-Cachán, Rovira, & Jaime, 2007). It showed that in the case of the high-linoleic salchichón, microorganisms remained inactive during post-HPP storage causing it to register the lowest counts and improved microbial stability. Other authors reached the same conclusions in different meat systems with inclusion of olive oil and grape seed oil correlated to higher unsaturated fatty acid contents, improved microbial inactivation (Jung et al., 2012; Kruk et al., 2014) and suggested the potential beneficial role of some unsaturated fatty acids in HPP inactivation of microorganisms in complex matrices. Remarkably, duck and goose fatty liver contains high concentrations of oleic acid (>50% total fatty acids composition) (Rukke, Fernandez, & Schüller, 2008). In light of the results introduced here, microbial inactivation in fatty duck liver by HPP could be facilitated thanks to the matrix composition. The results of Molin and Snygg (1967) and Ababouch et al. (1995) might thus potentially be due to an improved inactivation in given oils rather than a protective effect of the fatty acids of some other oils or a combined effect of protective or harmful fatty acids. These results, obtained with the natural flora of given food matrices, allow for hypothesis as to the impact of fatty acids on spore inactivation but will need to be confirmed for bacterial spores specifically. Further investigations are required to obtain a full mechanistic understanding of the role of fatty acids in HPP inactivation of spores.

In order to summarize the latest findings and compare bacterial spore inactivation in different food model systems based on their a_w, isokinetic lines and sample data points obtained at 600 MPa for a 5 $\log_{10}$ inactivation of *B. amyloliquefaciens* spores, one of the promising indicators for HPTS, are presented in dependency of a_w and temperature for given treatment times (Fig. 4). These results summarize and compare spore HPTS inactivation in different environments with variable a_w introduced in this review. Fig. 4 clearly highlights the complexity of the inactivation of spores by HPTS in real food systems. The inactivation kinetics are strongly influenced by the matrix and a_w alone cannot fully account for this difference.

HPTS proved to achieve a 5 $\log_{10}$ inactivation at 600 MPa in variable low a_w matrices with relatively low temperatures (90 °C). However, the required holding time at the lower temperatures and a given a_w was visibly matrix dependent, confirming the findings presented in this review. The variable baroprotective effect of the matrix was clear for T < 110 °C but less visible for higher temperature. Most interestingly, when looking at real food systems, it was noticeable that baby food behaved fairly closely to solute systems. On the other hand, fish in oil and brine systems exhibited a remarkable behavior for 10 and 5 min data points. Namely, in spite of a relatively lower a_w, the temperature needed to achieve inactivation of 5 $\log_{10}$ was lower than for baby food or solutes systems.

Overall, it could be concluded that HPP preservation of fat/oil containing matrices might be more challenging due to the formation of local (or global) low a_w refuges. However, not all fats/oils behave the same, and it was shown that a more systematic evaluation of the protective or deleterious role of different fatty acids as well as the impact of high pressure on the fatty acids profile needs to be better understood to predict inactivation in these matrices. Moreover, the membrane properties (hydrophobic/hydrophilic) of different microorganisms

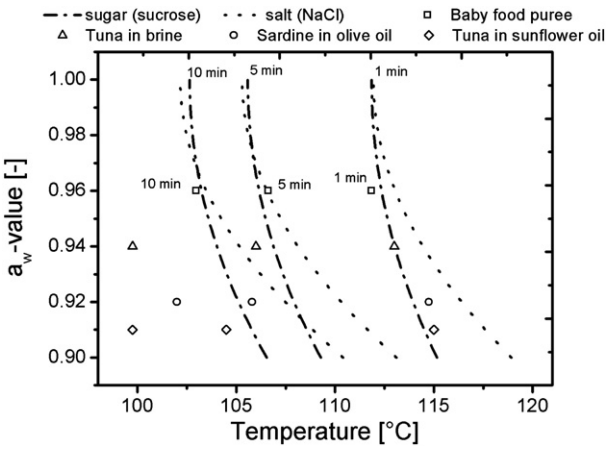

Fig. 4. Isokinetic lines for a 5 $\log_{10}$ inactivation of *B. amyloliquefaciens* spores in different model systems and experimental data in real food systems in dependency of a_w and temperature at given treatment times. Real food systems: tuna in brine (a_w 0.94); tuna in sunflower oil (0.91); sardine in olive oil (a_w 0.92) and baby food puree (a_w 0.96). NaCl [1.2–2.7 mol/L] and sucrose [0.83–1.7 mol/L]. Data derived from Sevenich et al. (2013); Sevenich, Kleinstueck, et al. (2014); and Sevenich, Thieme, Hecht, Rauh, and Knorr (2014). Isokinetics established based on the nth-order approach described in Section 3.

might as well affect their behavior in emulsified matrices and also need to be considered.

4. Conclusions and need for future work

This review summarized the multiple interactions and impacts of complex matrices and, in particular, of low or variable a_w on the microbial inactivation by HPP. HPP inactivation of microorganisms at low or variable a_w is reduced, but this limitation also applies to other preservation technologies. HPP could thus still support a reduction of the thermal intensity for these product categories.

The current state of knowledge of the inactivation mechanisms was reviewed. In particular, it was shown that:

- The impact of solutes on high pressure inactivation of microorganisms has to be considered at two levels:
  - The impact on the a_w. The reduced a_w alone cannot fully explain the baroprotection conferred by solutes. It is however clear that a reduction in a_w will contribute to stabilizing proteins against denaturation.
  - The individual properties of the solute and its properties in solution which might provide an additional protection against pressure induced inactivation of microorganisms, as was suggested for kosmotropic solutes.

Practically, the use of HPP for food matrices with high solute concentrations has already shown potential for specific applications. The first case of HPP processed commercial food product was fruit jams (Meidiya food factory Co.) in Japan (Horie, Kimura, & Hori, 1991) to inactivate yeasts, which highlighted the opportunity for microorganisms in highly concentrated solute environments (Cheftel, 1995; Yaldagard, Mortazavi, & Tabatabaie, 2008).

- The impact of the dehydration of food systems on the a_w and microorganism protection against HPP inactivation has led to only few applications for HPP preservation because of the limited amount of compression medium. Nonetheless, HPP treated dried food products exist already on the market for 10 years, such as cured and smoked sliced ham with a_w 0.89 and NaCl 4.60% content, suggesting that HPP preservation also carries potential for this product category. In fact, if only low initial contamination occurs, for example in cured and smoked ham, HPP could be valuable by inactivation of these limited amounts of microorganisms and a risk reduction, such as for *Listeria* free ham products. Additional wetting steps prior to HPP might also be considered for other dried products where a current safety gap exists and no other alternatives could be applied.
- The impact of fats and oils on the resistance of microorganisms to HPP seems to act in a similar way as for heat inactivation. Bacterial surfaces with hydrophobic or hydrophilic properties affect the shift to the aqueous or oil phase in emulsions, where the oil phase has a much lower local a_w and therefore lower inactivation. Also, individual fatty acids may support improved inactivation of microorganisms although no clear mechanism could be identified thus far. One might further note that only very few studies focused on the HPP inactivation of bacterial spores in fat or oil containing products. Bacterial spores may be introduced in a fat matrix by means of specific ingredients (e.g. herbs) and trigger a risk for the consumer or induce spoilage of the product if the conditions allow germination. The fact that fats have up to three times higher adiabatic heating than water might open opportunities for improved inactivation of spores by high pressure thermal sterilization and could support sterilization of fat containing matrices with a reduced thermal load.

This review underlines that the investigation of HPP microorganism inactivation mechanisms in low a_w matrices imposes to account for the matrix composition and not only the a_w. Inactivation kinetics in real food systems might significantly differ from kinetics in solutions at the same a_w. Single case studies are thus needed and the a_w is only one of several relevant parameters to consider. There may be a strong influence of the different ingredients in more complex food systems which could lead to unexpected results, such as, a lower temperature requirement for 5 $\log_{10}$ inactivation with long holding times in tuna in oil than in a baby food model (Fig. 4). This is particularly true for the low temperature/long holding time HPP combinations where the temperature contribution does not overrun matrices specificities. There, adding solutes or removing water might also change physico-chemical parameters of the matrix, such as, its microviscosity and solute's diffusivity which might in turn affect the inactivation kinetics of microorganisms.

Clear mechanistic understanding will need to be achieved and combined to adequate kinetic modeling to enable a satisfactory process control of pasteurization or sterilization and take advantage of the individualities offered by HPP in terms of microbial inactivation. Additionally, these process windows need to allow for a safety and/or quality improvement by comparison to conventional processing which should be assessed in terms of consumer acceptance and nutritional benefits.

Next, there is a clear need for equipment development to enable reaching the process conditions required for high pressure high temperature sterilization. While industrial production of HPP pasteurized foods reached over 500,000 t in 2014 (Tonello Samson, C. 2014, Hiperbaric, Spain, personal communication), the current industrial equipment does not allow for uniform temperature processing and thus sterilization at industrial-scale. Furthermore, when considering HPTS, a major challenge remains the definition of the best indicator strain as well as the accurate initial concentration in the respective food matrix. The well-established process windows for thermal processing are quite often based on extensive empirical data sets, which are still missing for HPP. Based on the multiplicity of the mechanisms at stake in different food matrices, it might also be interesting to consider product-specific strain selection for process validation.

Finally, future work will need to look at the impact of HPP on other food safety threats in complex matrices. Typically, many food borne diseases are linked to virus contamination, but only limited data for the inactivation by HPP exists. Another non-microbial hazard could come from prion proteins, which are extremely resistant to heat and pressure, but also in this topic, very few research papers were published. There again, variation in the food matrix composition could induce increased resistance which needs to be characterized.

Acknowledgments

The authors gratefully acknowledge the Nestlé Research Center, Lausanne for its financial support. This work was also partially financially supported by the European Commission (project PROMETHEUS, PROcess contaminants: Mitigation and Elimination Techniques for High food quality and their Evaluation Using Sensors & Simulation; FP7-KBBE-2010-4-265558).

References

Ababouch, L., Grimit, L., Eddafry, R., & Busta, F.F. (1995). Thermal inactivation kinetics of *Bacillus subtilis* spores suspended in buffer and in oils. *Journal of Applied Bacteriology*, *78*(6), 669–676.

Abe, F. (2013). Dynamic structural changes in microbial membranes in response to high hydrostatic pressure analyzed using time-resolved fluorescence anisotropy measurement. *Biophysical Chemistry*, *183*, 3–8.

Ahn, J., Lee, H. -Y., & Balasubramaniam, V.M. (2014). Inactivation of *Geobacillus stearothermophilus* spores in low-acid foods by pressure-assisted thermal processing. *Journal of the Science of Food and Agriculture*, http://dx.doi.org/10.1002/jsfa.6700.

Ananta, E., Heinz, V., & Knorr, D. (2005). Assessment of high pressure induced damage on *Lactobacillus rhamnosus* GG by flow cytometry. *Food Microbiology*, *21*(5), 567–577.

Ananta, E., Heinz, V., Schlüter, O., & Knorr, D. (2001). Kinetic studies on high-pressure inactivation of *Bacillus stearothermophilus* spores suspended in food matrices. *Innovative Food Science & Emerging Technologies*, *2*, 261–272.

Ardia, A. (2004). *Process considerations on the application of high pressure treatment at elevated temperature levels for food preservation.* (Ph.D thesis). Berlin: Berlin University of Technology.

Ardia, A., Knorr, D., & Heinz, V. (2004). Adiabatic heat modelling for pressure build-up during high-pressure treatment in liquid-food processing. *Food and Bioproducts Processing*, *82*(C1), 89–95.

Balasubramaniam, V., Farkas, D., & Turek, E. (2008). Preserving foods through high-pressure processing. *Food Technology*, *62*(11), 32–38.

Ballestra, P., Cruz, C., Antoine, M., Largeteau, A., Demazeau, G., Le Ba, D., et al. (1999). Thermal effect before high pressure treatment on the reduction of *Enterococcus faecalis* in duck fatty liver. In H. Ludwig (Ed.), *Advances in high pressure bioscience and biotechnology: proceedings of the International Conference on High Pressure Bioscience and Biotechnology, Heidelberg, August 30-September 3, 1998*. Berlin, Germany: Springer.

Barba, F.J., Esteve, M.J., & Frígola, A. (2012). High pressure treatment effect on physico-chemical and nutritional properties of fluid foods during storage: A review. *Comprehensive Reviews in Food Science and Food Safety*, *11*(3), 307–322.

Barbosa-Cánovas, G.V., & Vega-Mercado, H. (1996). *Dehydration of foods*. New York, NY: Chapman & Hall.

Bermúdez-Aguirre, D., & Barbosa-Cánovas, G. (2011). Recent advances in emerging non-thermal technologies. In J.M. Aguilera, R. Simpson, J. Welti-Chanes, D. Bermudez-Aguirre, & G. Barbosa-Canovas (Eds.), *Food engineering interfaces*. (pp. 285–323) New York: Springer.

Black, E.P., Koziol-Dube, K., Guan, D., Wei, J., Setlow, B., Cortezzo, D.E., et al. (2005). Factors influencing germination of *Bacillus subtilis* spores via activation of nutrient receptors by high pressure. *Applied and Environmental Microbiology*, *71*(10), 5879–5887.

Bolumar, T., Georget, E., & Mathys, A. (2014). High pressure processing (HPP) of foods and its combination with electron beam processing. In S. Pillai, & S. Shayanfar (Eds.), *Electron beam pasteurization and complementary food processing technologies*. Cambridge, UK: Woodhead Publishing 978-1-7-8242-100-9.

Bridgman, P.W. (1912). Water, in the liquid and five solid forms, under pressure. *Proceedings of the American Academy of Arts and Sciences*, *47*(13), 441–558.

Brown, A.D. (1978). Compatible solutes and extreme water stress in eukaryotic micro-organisms. *Advances in Microbial Physiology*, *17*, 181–242.

Buckow, R., & Heinz, V. (2008). High pressure processing — A database of kinetic information. *Chemie Ingenieur Technik*, *80*(8), 1081–1095.

Butz, P., Heinisch, O., & Tauscher, B. (1994). Hydrostatic high pressure applied to food sterilization III: Application to spices and spice mixtures. *High Pressure Research*, *12*(4–6), 239–243.

Capellas, M., Mor-Mur, M., Gervilla, R., Yuste, J., & Guamis, B. (2000). Effect of high pressure combined with mild heat or nisin on inoculated bacteria and mesophiles of goat's milk fresh cheese. *Food Microbiology*, *17*(6), 633–641.

Chapman, B., Winley, E., Fong, A., Hocking, A., Stewart, C., & Buckle, K. (2007). Ascospore inactivation and germination by high pressure processing is affected by ascospore age. *Innovative Food Science & Emerging Technologies*, *8*(4), 531–534.

Cheftel, J.C. (1995). Review: High pressure, microbial inactivation and food preservation. *Food Science and Technology International*, *1*, 75–90.

Cioni, P., & Strambini, G.B. (1994). Pressure effects on protein flexibility monomeric proteins. *Journal of Molecular Biology*, *242*(3), 291–301.

Clouston, J.G., & Wills, P.A. (1969). Initiation of germination and inactivation of *Bacillus pumilus* spores by hydrostatic pressure. *Journal of Bacteriology*, *97*(2), 684–690.

Considine, K.M., Kelly, A.L., Fitzgerald, G.F., Hill, C., & Sleator, R.D. (2008). High-pressure processing — Effects on microbial food safety and food quality. *FEMS Microbiology Letters*, *281*(1), 1–9.

Cruz, C., El Moueffak, A., Antoine, M., Montury, M., Demazeau, G., Largeteau, A., et al. (2003). Preservation of fatty duck liver by high pressure treatment. *International Journal of Food Science and Technology*, *38*(3), 267–272.

Degner, R. (2009). *pH-Messung*. Weinheim: Wiley-VCH.

Di Primo, C., Deprez, E., Hoa, G.H., & Douzou, P. (1995). Antagonistic effects of hydrostatic pressure and osmotic pressure on cytochrome P-450cam spin transition. *Biophysical Journal*, *68*(5), 2056–2061.

Distèche, A. (1959). pH measurement with a glass electrode withstanding 1500 kg/cm^2 hydrostatic pressure. *Review of Scientific Instruments*, *30*(6), 474–478.

Doona, C.J., & Feeherry, F.E. (2007). *High pressure processing of foods* (1st ed.). Blackwell Pub.

Dumay, E., Chevalier-Lucia, D., & Lopez-Pedemonte, T. (2010). High pressure and food conservation. In P. Sebert (Ed.), *Comparative high pressure biology* (pp. 85–121). Enfield, New Hampshire, USA: Science Publishers.

Duranton, F., Guillou, S., Simonin, H., Cheret, R., & de Lamballerie, M. (2012). Combined use of high pressure and salt or sodium nitrite to control the growth of endogenous microflora in raw pork meat. *Innovative Food Science & Emerging Technologies*, *16*, 373–380.

Eder, C., & Delgado, A. (2007). Interferometrische Messung der Dichte wässriger Lösungen unter Ultra-Hochdruck [Interferometric measurement of the density of aqueous solutions under ultra-high hydrostatic pressure]. *tm — Technisches Messen. Vol. 74*. (pp. 45).

Eisenbrand, G. (2005). Safety assessment of high pressure treated foods — Opinion of the Senate Commission on Food Safety (SKLM) of the German Research Foundation (DFG) — (shortened version). *Molecular Nutrition & Food Research*, *49*(12), 1168–1174.

El Moueffak, A., Cruz, C., Antoine, M., Montury, M., Demazeau, G., Largeteau, A., et al. (1994). Stabilisation of fatty duck liver by high pressure treatment. *High Pressure Research*, *12*(4–6), 245–250.

El Moueffak, A., Cruz, C., Antoine, M., Montury, M., Demazeau, G., Largeteau, A., et al. (2001). Stabilization of duck fatty liver by high pressure treatment. Inactivation of *Enterococcus faecalis*. *Sciences des Aliments*, *21*(1), 71–76.

Espuña, SA (2014). High Pressure. Accessed on 16/05, 2014. http://www.espuna.co.uk/en/innovation/high-pressure.aspx

Furukawa, S., & Hayakawa, I. (2000). Investigation of desirable hydrostatic pressure required to sterilize *Bacillus stearothermophilus* IFO 12550 spores and its sterilization properties in glucose, sodium chloride and ethanol solutions. *Food Research International*, *33*, 901–905.

Gaillard, S., Leguerinel, I., & Mafart, P. (1998). Model for combined effects of temperature, pH and water activity on thermal inactivation of *Bacillus cereus* spores. *Journal of Food Science*, *63*(5), 887–889.

Garriga, M., Grèbol, N., Aymerich, T., Monfort, J.M., & Hugas, M. (2004). Microbial inactivation after high-pressure processing at 600 MPa in commercial meat products over its shelf life. *Innovative Food Science & Emerging Technologies*, *5*(4), 451–457.

Gayán, E., Condón, S., Álvarez, I., Nabakabaya, M., & Mackey, B. (2013). The effect of pressure-induced changes in the ionisation equilibria of buffers on inactivation of *Escherichia coli* and *Staphylococcus aureus* by high hydrostatic pressure. *Applied and Environmental Microbiology*, *79*(13), 4041–4047.

Georget, E., Kapoor, S., Winter, R., Reineke, K., Song, Y., Callanan, M., et al. (2014). *In situ* investigation of *Geobacillus stearothermophilus* spore germination and inactivation mechanisms under moderate high pressure. *Food Microbiology*, *41*, 8–18.

Georget, E., Reineke, K., Heinz, V., Knorr, D., Ananta, E., & Mathys, A. (2013). Spore inactivation mechanisms during industrial food and equipment sterilization. In D.R. Heldman, M.B. Wheeler, & D.G. Hoover (Eds.), *Encyclopedia of biotechnology in agriculture and food* (pp. 1–6). Taylor & Francis.

Goodridge, L.D., Willford, J., & Kalchayanand, N. (2006). Destruction of *Salmonella Enteriditis* inoculated onto raw almonds by high hydrostatic pressure. *Food Research International*, *39*(4), 408–412.

Gould, G.W. (1985). Present state of knowledge of a_w effects on microorganisms. In D. Simatos, J.L. Multon, & North Atlantic Treaty Organization. Scientific Affairs Division (Eds.), *Properties of water in foods: In relation to quality and stability* (pp. 229–245). The Netherlands, Dordrecht: M. Nijhoff.

Gould, G.W., Measures, J.C., Wilkie, D.R., & Meares, P. (1977). Water relations in single cells [and discussion]. *Philosophical Transactions of the Royal Society of London B, Biological Sciences*, *278*(959), 151–166.

Gould, G.W., & Sale, A.J.H. (1970). Initiation of germination of bacterial spores by hydrostatic pressure. *Journal of General Microbiology*, *60*, 335.

Grant, W.D. (2004). Life at low water activity. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences*, *359*(1448), 1249–1267.

Grauwet, T., Plancken, I.V. d, Vervoort, L., Hendrickx, M.E., & Loey, A.V. (2010). Mapping temperature uniformity in industrial scale HP equipment using enzymatic pressure–temperature–time indicators. *Journal of Food Engineering*, *98*(1), 93–102.

Grauwet, T., Rauh, C., Van der Plancken, I., Vervoort, L., Hendrickx, M., Delgado, A., et al. (2012). Potential and limitations of methods for temperature uniformity mapping in high pressure thermal processing. *Trends in Food Science & Technology*, *23*(2), 97–110.

Gupta, R., & Balasubramaniam, V.M. (2012). High-pressure processing of fluid foods. In P.J. Cullen, B.K. Tiwari, & V. Valdramidis (Eds.), *Novel thermal and non-thermal technologies for fluid foods* (pp. 109–133) (1st ed.). Elsevier Science.

Harris, L. (2013). *Improving the safety and quality of nuts*. Cambridge, UK: Woodhead Publishing.

Hayert, M., Perrier-Cornet, J. -M., & Gervais, P. (1999). A simple method for measuring the pH of acid solutions under high pressure. *The Journal of Physical Chemistry. A*, *103*, 1785–1789.

Hayman, M.M., Kouassi, G.K., Anantheswaran, R.C., Floros, J.D., & Knabel, S.J. (2008). Effect of water activity on inactivation of *Listeria monocytogenes* and lactate dehydrogenase during high pressure processing. *International Journal of Food Microbiology*, *124*(1), 21–26.

Heinz, V. (1997). *Wirkung hoher hydrostatischer Drücke auf das Absterbe- und Keimungsverhalten sporenbildender Bakterien am Beispiel von Bacillus subtilis ATCC 9372*. (Ph.D.). Berlin: Berlin University of Technology.

Heinz, V., & Buckow, R. (2010). Food preservation by high pressure. *Journal für Verbraucherschutz und Lebensmittelsicherheit*, *5*(1), 73–81.

Heinz, V., & Knorr, D. (1996). High pressure inactivation kinetics of *Bacillus subtilis* cells by a three-state-model considering distribution resistance mechanisms. *Food Biotechnology*, *10*(2), 149–161.

Heinz, V., & Knorr, D. (1998). High pressure germination and inactivation kinetics of bacterial spores. In N.S. Isaacs (Ed.), *High pressure food science, bioscience and chemistry* (pp. 435–441). Cambridge: The Royal Society of Chemistry.

Heinz, V., & Knorr, D. (2002). Effects of high pressure on spores. In M.E.G. Hendrickx, & D. Knorr (Eds.), *Ultra high pressure treatments of foods* (pp. 77–114). New York: Kluwer Academic/Plenum Publishers.

Hendrickx, M., & Knorr, D. (2002). *Ultra high pressure treatment of foods*. New York: Kluwer Academic/Plenum Publishers.

Heremans, K. (2002). The effects of high pressure on biomaterials. In M. Hendrickx, & D. Knorr (Eds.), *Ultra high pressure treatment of foods* (pp. 23–46). New York: Kluwer Academic/Plenum Publishers.

Hite, B.H. (1899). The effect of pressure in the preservation of milk. *Bulletin of West Virginia University of Agriculture Experimental Station Morgantown*, *58*, 15–35.

Hofstetter, S., Winter, R., McMullen, L.M., & Gänzle, M.G. (2013). *In situ* determination of *Clostridium* endospore membrane fluidity during pressure-assisted thermal processing in combination with nisin or reutericyclin. *Applied and Environmental Microbiology*, *79*(6), 2103–2106.

Hogan, E., Kelly, A.L., & Sun, D. -W. (2005). High pressure processing of foods: An overview. In D. -W. Sun (Ed.), *Emerging technologies for food processing* (pp. 3–32). London: Academic Press.

Hoover, D.G., Metrick, C., Papineau, A.M., Farkas, D.F., & Knorr, D. (1989). Biological effects of high hydrostatic pressure on food microorganisms. *Food Technology*, *43*(9), 99–107.

Horie, Y., Kimura, K., & Hori, K. (1991). Development of a new fruit processing method by high hydrostatic pressure. *Journal of the Agricultural Chemical Society of Japan*, *65*(10), 1469–1474.

Huang, H. -W., Lung, H. -M., Yang, B.B., & Wang, C. -Y. (2014). Responses of microorganisms to high hydrostatic pressure processing. *Food Control*, *40*, 250–259.

Hugas, M., Garriga, M., & Monfort, J.M. (2002). New mild technologies in meat processing: High pressure as a model technology. *Meat Science*, *62*(3), 359–371.

Illinois Institute of Technology (2009). NCFST receives regulatory acceptance of novel food sterilization process. Accessed on 26/05, 2014. http://www.iit.edu/departments/pr/mediaroom/article_viewer_db.php?articleID=355

Jofré, A., Aymerich, T., Grèbol, N., & Garriga, M. (2009). Efficiency of high hydrostatic pressure at 600 MPa against food-borne microorganisms by challenge tests on convenience meat products. *LWT - Food Science and Technology*, *42*(5), 924–928.

Juliano, P., Knoerzer, K., Fryer, P.J., & Versteeg, C. (2009). *C. botulinum* inactivation kinetics implemented in a computational model of a high-pressure sterilization process. *Biotechnology Progress*, *25*(1), 163–175.

Jung, Y. -K., Jung, S., Lee, H. -J., Kang, M. -G., Lee, S. -K., Kim, Y. -J., et al. (2012). Effect of high pressure after the addition of vegetable oil on the safety and quality of beef loin. *Korean Journal for Food Science of Animal Resources*, *32*(1), 68–76.

Kessler, H.G. (2002). *Food and bio process engineering: Dairy technology* (5th ed.). Munich, Germany: Kessler, A.

Klotz, B., Manas, P., & Mackey, B.M. (2010). The relationship between membrane damage, release of protein and loss of viability in *Escherichia coli* exposed to high hydrostatic pressure. *International Journal of Food Microbiology*, *137*(2–3), 214–220.

Knoerzer, K., Buckow, R., Chapman, B., Juliano, P., & Versteeg, C. (2010). Carrier optimisation in a pilot-scale high pressure sterilisation plant — An iterative CFD approach employing an integrated temperature distributor (ITD). *Journal of Food Engineering*, *97*(2), 199–207.

Knoerzer, K., Juliano, P., Gladman, S., Versteeg, C., & Fryer, P. (2007). A computational model for temperature and sterility distributions in a pilot-scale high-pressure high-temperature process. *American Institute of Chemical Engineers Journal*, *53*(11), 2996–3010.

Knorr, D., Froehling, A., Jaeger, H., Reineke, K., Schlueter, O., & Schoessler, K. (2011). Emerging technologies in food processing. *Annual Review of Food Science and Technology*, *2*, 203–235.

Knorr, D., Reineke, K., Mathys, A., Heinz, V., & Buckow, R. (2011). High-pressure-induced effects on bacterial spores, vegetative microorganisms, and enzymes. In J.M. Aguilera, R. Simpson, J. Welti-Chanes, D. Bermudez-Aguirre, & G. Barbosa-Canovas (Eds.), *Food engineering interfaces* (pp. 325–340). New York: Springer.

Koseki, S., & Yamamoto, K. (2007a). Water activity of bacterial suspension media unable to account for the baroprotective effect of solute concentration on the inactivation of *Listeria monocytogenes* by high hydrostatic pressure. *International Journal of Food Microbiology*, *115*(1), 43–47.

Koseki, S., & Yamamoto, K. (2007b). A novel approach to predicting microbial inactivation kinetics during high pressure processing. *International Journal of Food Microbiology*, *116*(2), 275–282.

Kruk, Z.A., Kim, H.J., Kim, Y.J., Rutley, D.L., Jung, S., Lee, S.K., et al. (2014). Combined effects of high pressure processing and addition of soy sauce and olive oil on safety and quality characteristics of chicken breast meat. *Asian-Australasian Journal of Animal Sciences*, *27*(2), 256–265.

Lee, E.L., & Gilbert, C.A. (1918). On the application of the mass law to the process of disinfection — being a contribution to the 'mechanistic theory' as opposed to the 'vitalistic theory'. *Journal of Physical Chemistry*, *22*, 348–372.

Lenovich, L. (1987). Survival and death of microorganims as influenced by water activity. In L.B. Rockland, L.B. Beuchat, & Institute of Food Technologists (Eds.), *Water activity: Theory and application to food* (pp. 119–136).

Margosch, D., Ehrmann, M.A., Buckow, R., Heinz, V., Vogel, R.F., & Gänzle, M.G. (2006). High-pressure-mediated survival of *Clostridium botulinum* and *Bacillus amyloliquefaciens* endospores at high temperature. *Applied and Environment Microbiology*, *72*(5), 3476–3481.

Margosch, D., Gänzle, M.G., Ehrmann, M.A., & Vogel, R.F. (2004). Pressure inactivation of *Bacillus* endospores. *Applied and Environment Microbiology*, *70*(12), 7321–7328.

Mathys, A. (2008). *Inactivation mechanisms of Geobacillus and Bacillus spores during high pressure thermal sterilization.* (Ph.D. thesis). Berlin, Germany: Technische Universität Berlin (Accessible at:http://www.worldcat.org/wcpa/oclc/244076025?page=frame&url=http%3A%2F%2Fopus.kobv.de%2Ftuberlin%2Fvolltexte%2F2008%2F1873%2Fpdf%2Fmathys_alexander.pdf%26checksum%3D550bd78c2d2ba47c77b13a467d5c0c2c&title=&linktype=digitalObject&detail=).

Mathys, A., Chapman, B., Bull, M., Heinz, V., & Knorr, D. (2007). Flow cytometric assessment of *Bacillus* spore response to high pressure and heat. *Innovative Food Science and Emerging Technologies*, *8*(4), 519–527.

Mathys, A., Kallmeyer, R., Heinz, V., & Knorr, D. (2008). Impact of dissociation equilibrium shift on bacterial spore inactivation by heat and pressure. *Food Control*, *19*(12), 1165–1173.

Mathys, A., Reineke, K., Heinz, V., & Knorr, D. (2009). High pressure thermal sterilization — Development and application of temperature controlled spore inactivation studies. *High Pressure Research*, *29*(1), 3–7.

Metrick, C., Hoover, D.G., & Farkas, D.F. (1989). Effects of high hydrostatic pressure on heat-resistant and heat-sensitive strains of *Salmonella*. *Journal of Food Science*, *54*(6), 1547–1549.

Meyer, R. S. (2000). Ultra high pressure, high temperature food preservation process. Patent number: WO/2000/015053.

Min, S., Sastry, S.K., & Balasubramaniam, V.M. (2010). Compressibility and density of select liquid and solid foods under pressures up to 700 MPa. *Journal of Food Engineering*, *96*(4), 568–574.

Molin, N., & Snygg, B.G. (1967). Effect of lipid materials on heat resistance of bacterial spores. *Applied Microbiology*, *15*(6), 1422–1426.

Molina-Gutierrez, A., Rademacher, B., Gänzle, M.G., & Vogel, R.F. (2002). Effect of sucrose and sodium chloride on the survival and metabolic activity of *Lactococcus lactis* under high-pressure conditions. In R. Hayashi (Ed.), *Trends in high pressure bioscience and biotechnology* (pp. 295–302). Amsterdam, The Netherlands: Elsevier.

Molina-Gutierrez, A., Stippl, V.M., Delgado, A., Gänzle, M.G., & Vogel, R.F. (2002). *In situ* determination of the intracellular pH of *Lactococcus lactis* and *Lactobacillus plantarum* during pressure treatment. *Applied and Environmental Microbiology*, *68*(9), 4399–4406.

Molina-Höppner, A., Doster, W., Vogel, R.F., & Gänzle, M.G. (2004). Protective effect of sucrose and sodium chloride for *Lactococcus lactis* during sublethal and lethal high-pressure treatments. *Applied and Environmental Microbiology*, *70*(4), 2013–2020.

Morales, P., Calzada, J., Rodriguez, B., de Paz, M., Gaya, P., & Nunez, M. (2006). Effect of cheese water activity and carbohydrate content on the barotolerance of *Listeria monocytogenes* Scott A. *Journal of Food Protection*, *69*, 1328–1333.

Moussa, M., Perrier-Cornet, J. -M., & Gervais, P. (2006). Synergistic and antagonistic effects of combined subzero temperature and high pressure on inactivation of *Escherichia coli*. *Applied and Environmental Microbiology*, *72*(1), 150–156.

Murrell, W.G., & Scott, W.J. (1966). The heat resistance of bacterial spores at various water activities. *Journal of General Microbiology*, *43*(3), 411–425.

Neetoo, H., Pizzolato, T., & Chen, H. (2009). Elimination of *Escherichia coli* O157:H7 from Alfalfa seeds through a combination of high hydrostatic pressure and mild heat. *Applied and Environmental Microbiology*, *75*(7), 1901–1907.

Neetoo, H., Ye, M., & Chen, H. (2008). Potential application of high hydrostatic pressure to eliminate *Escherichia coli* O157:H7 on alfalfa sprouted seeds. *International Journal of Food Microbiology*, *128*(2), 348–353.

Oxen, P., & Knorr, D. (1993). Baroprotective effects of high solute concentrations against inactivation of *Rhodotorula rubra*. *LWT - Food Science and Technology*, *26*, 220–223.

Paidhungat, M., Setlow, B., Daniels, W.B., Hoover, D., Papafragkou, E., & Setlow, P. (2002). Mechanisms of induction of germination of *Bacillus subtilis* spores by high pressure. *Applied and Environmental Microbiology*, *68*(6), 3172–3175.

Palou, E., López-Malo, A., Barbosa-Cánovas, G.V., Welti-Chanes, J., & Swanson, B.G. (1997). High hydrostatic pressure as a hurdle for *Zygosaccharomyces bailii* inactivation. *Journal of Food Science*, *62*(4), 855–857.

Patazca, E., Koutchma, T., & Balasubramaniam, V.M. (2007). Quasi-adiabatic temperature increase during high pressure processing of selected foods. *Journal of Food Engineering*, *80*(1), 199–205.

Peleg, M., & Cole, M.B. (1998). Reinterpretation of microbial survival curves. *Critical Reviews in Food Science*, *38*(5), 353–380.

Peñas, E., Gómez, R., Frías, J., & Vidal-Valverde, C. (2008). Application of high-pressure treatment on alfalfa (*Medicago sativa*) and mung bean (*Vigna radiata*) seeds to enhance the microbiological safety of their sprouts. *Food Control*, *19*(7), 698–705.

Priev, A., Almagor, A., Yedgar, S., & Gavish, B. (1996). Glycerol decreases the volume and compressibility of protein interior. *Biochemistry*, *35*, 2061–2066.

Quinlan, R.J., & Reinhart, G.D. (2005). Baroresistant buffer mixtures for biochemical analyses. *Analytical Biochemistry*, *341*(1), 69–76.

Raffalli, J., Rosec, J., Carlez, A., Dumay, E., Richard, N., & Cheftel, J. (1994). High pressure stress and inactivation of *Listeria innocua* in inoculated dairy cream. *Sciences des Aliments*, *14*, 349–358.

Rajan, S., Ahn, J., Balasubramaniam, V.M., & Yousef, A.E. (2006). Combined pressure-thermal inactivation kinetics of *Bacillus amyloliquefaciens* spores in egg patty mince. *Journal of Food Protection*, *69*(4), 853–860.

Ramaswamy, H.S., Jin, H., & Zhu, S. (2009). Effects of fat, casein and lactose on high-pressure destruction of *Escherichia coli* K12 (ATCC-29055) in milk. *Food and Bioproducts Processing*, *87*(1), 1–6.

Rasanayagam, V., Balasubramaniam, V.M., Ting, E., Sizer, C.E., Bush, C., & Anderson, C. (2003). Compression heating of selected fatty food materials during high-pressure processing. *Journal of Food Science*, *68*(1), 254–259.

Raso, J., Góngora-Nieto, M.M., Barbosa-Cánovas, G.V., & Swanson, B.G. (1998). Influence of several environmental factors on the initiation of germination and inactivation of *Bacillus cereus* by high hydrostatic pressure. *International Journal of Food Microbiology*, *44*(1–2), 125–132.

Rastogi, N.K., Raghavarao, K.S., Balasubramaniam, V.M., Niranjan, K., & Knorr, D. (2007). Opportunities and challenges in high pressure processing of foods. *Critical Reviews in Food Science and Nutrition*, *47*(1), 69–112.

Rauh, C., Baars, A., & Delgado, A. (2009). Uniformity of enzyme inactivation in a short-time high-pressure process. *Journal of Food Engineering*, *91*(1), 154–163.

Reineke, K. (2012). *Mechanisms of Bacillus spore germination and inactivation during high pressure processing.* (Ph.D. thesis). Berlin, Germany: Technischen Universität Berlin (Accessible at: http://www.opus4.kobv.de/opus4-tuberlin/files/3659/reineke_kai.pdf).

Reineke, K., Doehner, I., Schlumbach, K., Baier, D., Mathys, A., & Knorr, D. (2011). The different pathways of spore germination and inactivation in dependence of pressure and temperature. *Innovative Food Science and Emerging Technologies*, *13*, 31–41.

Reineke, K., Mathys, A., Heinz, V., & Knorr, D. (2013). Mechanisms of endospore inactivation under high pressure. *Trends in Microbiology*, *21*(6), 296–304.

Reineke, K., Mathys, A., & Knorr, D. (2011a). The impact of high pressure and temperature on bacterial spores: inactivation mechanisms of *Bacillus subtilis* above 500 MPa. *Journal of Food Science*, *76*(3), 189–197.

Reineke, K., Mathys, A., & Knorr, D. (2011b). Shift of pH-value during thermal treatments in buffer solutions and selected foods. *International Journal of Food Properties*, *14*(4), 870–881.

Reps, A., Kolakowski, P., & Dajnowiec, F. (1998). Effects of high pressure on lipid oxidation in fish. In N.S. Isaacs (Ed.), *High pressure food science, bioscience and chemistry* (pp. 284–288). Cambridge, England: Royal Society of Chemistry.

Robert Koch Institute. (2011). Report: Final presentation and evaluation of epidemiological findings in the EHEC O104:H4 outbreak, Germany 2011. In RKI Department for Infectious Disease Epidemiology Division 35 (Ed.). Berlin.

Ruan, K., Xu, C., Li, T., Li, J., Lange, R., & Balny, C. (2003). The thermodynamic analysis of protein stabilization by sucrose and glycerol against pressure-induced unfolding. *European Journal of Biochemistry*, *270*(8), 1654–1661.

Rubio, B., Martínez, B., García-Cachán, M.D., Rovira, J., & Jaime, I. (2007). The effects of high pressure treatment and of storage periods on the quality of vacuum-packed "salchichón" made of raw material enriched in monounsaturated and polyunsaturated fatty acids. *Innovative Food Science & Emerging Technologies*, *8*(2), 180–187.

Rukke, E.O., Fernandez, X., & Schüller, R.B. (2008). Comparison of rheological properties in fatty liver from duck and goose by oscillatory plate–plate rheometer measurements. In Nordic Rheology Society (Ed.), *Annual transactions of the nordic rheology society. Vol. 16.* (pp. 57–62).

Schmidt, S.J., & Fontana, A.J. (2008). Appendix E: Water activity values of select food ingredients and products. In G.V. Barbosa Canovas, A.J. Fontana, S.J. Schmidt, & T.P. Labuza (Eds.), *Water activity in foods: Fundamentals and applications*. Oxford, UK: Blackwell Publishing Ltd.

Senhaji, A.F., & Loncin, M. (1977). The protective effect of fat on the heat resistance of bacteria (I). *International Journal of Food Science & Technology*, *12*(3), 203–216.

Sevenich, R., Bark, F., Crews, C., Anderson, W., Riddellova, K., Hradecky, J., et al. (2013). Effect of high pressure thermal sterilization on the formation of food processing contaminants. *Innovative Food Science and Emerging Technologies*, *20*, 42–50.

Sevenich, R., Bark, F., Kleinstueck, E., Crews, C., Pye, C., Hradecky, J., et al. (2015). The impact of high pressure thermal sterilization on the microbiological stability and formation of food processing contaminants in selected fish systems and baby food puree at pilot scale. *Food Control*, http://dx.doi.org/10.1016/j.foodcont.2014.09.050.

Sevenich, R., Kleinstueck, E., Crews, C., Anderson, W., Pye, C., Riddellova, K., et al. (2014). High-pressure thermal sterilization: Food safety and food quality of baby food puree. *Journal of Food Science*, *79*(2), M230–M237.

Sevenich, R., Thieme, E., Hecht, P., Rauh, C., & Knorr, D. (2014). Baroprotective effect of solutes against the high pressure inactivation of *Bacillus amyloliquefaciens* spores. *Poster abstract. At IFT Annual Meeting 2014, New Orleans, LA, USA — June 21–24.*

Simpson, R.K., & Gilmour, A. (1997). The effect of high hydrostatic pressure on *Listeria monocytogenes* in phosphate-buffered saline and model food systems. *Journal of Applied Microbiology*, *83*(2), 181–188.

Smelt, J.P. (1998). Recent advances in the microbiology of high pressure processing. *Trends in Food Science & Technology*, *9*, 152–158.

Smelt, J.P., Hellemons, J.C., & Patterson, M. (2001). Effects of high pressure on vegetative microorganisms. In M. Hendrickx, & D. Knorr (Eds.), *Ultra high pressure treatments of foods* (pp. 55–76). New York: Kluwer Academic.

Sperber, W.H. (1983). Influence of water activity on food borne bacteria — A review. *Journal of Food Protection*, *46*(142).

Stippl, V.M., Delgado, A., & Becker, T.M. (2002). Optical method for the *in-situ* measurement of the pH-value during high pressure treatment of foods. *High Pressure Research*, *22*, 757–761.

Stippl, V.M., Delgado, A., & Becker, T.M. (2004). Development of a method for the optical in-situ determination of pH value during high-pressure treatment of fluid food. *Innovative Food Science & Emerging Technologies*, *5*, 285–292.

Szczawinski, J., Szczawinski, M., Stanczack, B., Fonberg-Broczek, M., & Arabas, J. (1997). Effect of high pressure on survival of *Listeria monocytogenes* in ripened sliced cheese at ambient temperature. In K. Heremans, & C. Balny (Eds.), *High pressure research in the biosciences and biotechnology: proceedings of the XXXIVth Meeting of the European High Pressure Research Group, September 1–5, 1996*. Leuven, Belgium: Leuven University Press.

Taormina, P.J. (2010). Implications of salt and sodium reduction on microbial food safety. *Critical Reviews in Food Science and Nutrition*, *50*(3), 209–227.

Ting, E., Balasubramaniam, V.M., & Raghubeer, E. (2002). Determining thermal effects in high pressure processing. *Journal of Food Technology*, *56*(2), 31–35.

Toepfl, S., Mathys, A., Heinz, V., & Knorr, D. (2006). Review: potential of high hydrostatic pressure and pulsed electric fields for energy efficient and environmentally friendly food processing. *Food Reviews International*, *22*(4), 405–423.

Troller, J. (1987). Adaptation and growth of microorganisms in environments with reduced water activity. In L.B. Rockland, L.B. Beuchat, & Institute of Food Technologists (Eds.), *Water activity: Theory and application to food* (pp. 101–118).

Van Boekel, M.A.J.S. (2010). *Kinetic modeling of reactions in foods*. Taylor & Francis.

Van Doren, J., Dennis, S., Brandt, M., & Solomotis, M.D. (2013). FDA draft risk profile: pathogens and filth in spices. *Center for food safety and applied nutrition*. Food and Drug Administration — U.S. Department of Health and Human Services.

Van Opstal, I., Vanmuysen, S.C., & Michiels, C.W. (2003). High sucrose concentration protects *E. coli* against high pressure inactivation but not against high pressure sensitization to the lactoperoxidase system. *International Journal of Food Microbiology*, *88*(1), 1–9.

Voigt, D.D., Chevalier, F., Qian, M.C., & Kelly, A.L. (2010). Effect of high-pressure treatment on microbiology, proteolysis, lipolysis and levels of flavour compounds in mature blue-veined cheese. *Innovative Food Science and Emerging Technologies*, *11*(1), 68–77.

Watson, H.E. (1908). A note on the variation of the rate of disinfection with change in the concentration of the disinfectant. *Journal of Hygiene*, *8*, 536.

Wiencek, K.M., Klapes, N.A., & Foegeding, P.M. (1990). Hydrophobicity of *Bacillus* and *Clostridium* spores. *Applied and Environment Microbiology*, *56*(9), 2600–2605.

Willford, J., Mendonca, A., & Goodridge, L.D. (2008). Water pressure effectively reduces *Salmonella enterica serovar enteritidis* on the surface of raw almonds. *Journal of Food Protection*, *71*(4), 825–829.

Wimalaratne, S., & Farid, M. (2008). Pressure assisted thermal sterilization. *Food and Bioproducts Processing*, *86*(4), 312–316.

Winter, R., & Jeworrek, C. (2009). Effect of pressure on membranes. *Soft Matter*, *5*(17), 3157–3173.

Wuytack, E.Y., Boven, S., & Michiels, C.W. (1998). Comparative study of pressure-induced germination of *Bacillus subtilis* spores at low and high pressures. *Applied and Environmental Microbiology*, *64*(9), 3220–3224.

Wuytack, E.Y., Soons, J., Poschet, F., & Michiels, C.W. (2000). Comparative study of pressure- and nutrient-induced germination of *Bacillus subtilis* spores. *Applied and Environmental Microbiology*, *66*(1), 257–261.

Yaldagard, M., Mortazavi, S.A., & Tabatabaie, F. (2008). The principles of ultra high pressure technology and its application in food processing/preservation: A review of microbiological and quality aspects. *African Journal of Biotechnology*, *7*(16), 2739–2767.

Zancan, P., & Sola-Penna, M. (2005). Trehalose and glycerol stabilize and renature yeast inorganic pyrophosphatase inactivated by very high temperatures. *Archives of Biochemistry and Biophysics*, *444*(1), 52–60.

Zhang, Y., & Cremer, P.S. (2006). Interactions between macromolecules and ions: the Hofmeister series. *Current Opinion in Chemical Biology*, *10*(6), 658–663.